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Exhaled Markers of Pulmonary Disease

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INTRODUCTION

There has recently been an explosion of interest in the analysis of breath constituents as a way of monitoring inflammation and oxidative stress in the lungs. Here we review the use of exhaled breath analysis in the diagnosis and monitoring of lung disease. Although most studies have focused on exhaled nitric oxide (NO), recently several other volatile gases (carbon monoxide, ethane, pentane) have also been used. In addition, several endogenous substances (inflammatory mediators, cytokines, oxidants) may be detected in expired breath condensates, opening up new perspectives for exhaled breath analysis.

Many lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, cystic fibrosis, and interstitial lung disease, involve chronic inflammation and oxidative stress. Yet these are not measured directly in routine clinical practice because of the difficulties in monitoring inflammation. In asthma fiberoptic bronchial biopsies have become the "gold standard" for measuring inflammation in the airway wall, but this is an invasive procedure that is not suitable for routine clinical practice and cannot be repeated often. It is also unsuitable for use in children and patients with severe disease. Symptoms may not accurately reflect the extent of underlying inflammation because of differences in perception and masking by bronchodilators in airway disease. In asthma measurement of airway hyperresponsiveness by histamine or methacholine challenge has been used as a surrogate marker of inflammation, but interpretation may be confounded by the use of bronchodilator therapy. Furthermore, it is difficult to perform this measurement in children and in patients with severe disease. This has led to the use of induced sputum to detect inflammation. This method is relatively reproducible and allows the quantification of inflammatory cells and mediators (1). However, this technique is somewhat invasive as it involves inhalation of hypertonic saline, which may induce coughing and bronchoconstriction, and it is difficult to use in small children. Furthermore, the technique itself induces an inflammatory response so that it is not possible to re-

(Received in original form September 5, 2000 and in revised form January 24, 2001)

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Am J Respir Crit Care Med Vol 163. pp 1693–1722, 2001
Internet address: www.atsjournals.org

PM3006723943

peat measurements in less than 24 h (2). The need to monitor inflammation in the lungs has led to the exploration of exhaled gases and condensates. Noninvasive monitoring may assist in differential diagnosis of pulmonary diseases, assessment of disease severity and response to treatment. Because these techniques are completely noninvasive, they can be used repeatedly to give information about kinetics, they can be used in patients with severe disease, which has been previously difficult to monitor, and they can be used to monitor disease in children, including infants. Breath analysis is currently a research procedure, but there is increasing evidence that it may have an important place in the diagnosis and management of lung diseases in the future (3). This will drive the development of cheaper and more convenient analyzers, which can be used in a hospital and later in a family practice setting, then eventually to the development of personal monitoring devices for use by patients.

NITRIC OXIDE

NO is the most extensively studied exhaled marker and abnormalities in exhaled NO have been documented in several lung diseases (3), particularly asthma (4–6).

Source of NO in Exhaled Air

Nitric oxide synthases. Endogenous NO is derived from L-arginine by the enzyme NO synthase (NOS), of which at least three distinct isoforms exist (7) (Figure 1, *panel A*). Two of these enzymes are constitutively expressed and are activated by small rises in intracellular calcium concentration, secondary to cell activation. Neuronal NOS (NOS1, nNOS) is predominantly expressed in neurones and endothelial NOS (NOS3, eNOS) mainly in endothelial cells, although other cell types also express both of these isoforms. A third enzyme is inducible (NOS2, iNOS), has a much greater level of activity, and is independent of calcium concentration. NOS2 may be induced by inflammatory cytokines, endotoxin, and viral infections and may show increased expression in inflammatory diseases (8–10). Genetic polymorphisms of all three isoforms of NOS have been detected. Surprisingly, associations have been found between polymorphisms in the NOS1 gene and asthma in Caucasian populations (11, 12). In patients with mild asthma there is a significant association between the length of the AAT repeat polymorphism in intron 20 of the NOS1 gene and exhaled NO levels (13).

Cellular sources in airways. The cellular source of NO gas in the lower respiratory tract is not yet certain. Studies with perfused porcine lungs suggest that exhaled NO originates at the alveolar surface, rather than from the pulmonary circulation (14), and it may be derived from NOS3 expressed in the alveolar walls of normal lungs. Studies in ventilated perfused lungs of guinea pigs have shown that exhaled NO is reduced during perfusion with calcium-free solutions, suggesting that NO is derived from a constitutive NOS, which is calcium-dependent (15). Airway epithelial cells may express both NOS3 and NOS1 and therefore may contribute to NO in the lower respiratory tract (16, 17). There is some expression of NOS2 even in airway epithelial cells from normal subjects (18), and NOS2 appears to be an important isoform contributing to exhaled NO in healthy mice (19). In inflammatory diseases such as asthma it is likely that the increase in exhaled NO reflects further induction of NOS2 in response to inflammatory signals such as proinflammatory cytokines. Indeed, increased NOS activity has been demonstrated in lung tissue of patients with asthma, cystic fibrosis, and obliterative bronchiolitis (20). In asthmatic patients there is evidence for increased expression of NOS2 in airway epithelial cells (21), and this is likely to be due to increased transcription mediated via the transcription factors STAT-1 and nuclear factor-κB (NF-κB), and increased availability of L-arginine (22, 23). Proinflammatory cytokines induce the expression of NOS2 in cultured human airway epithelial cells (24, 25), and it is likely that these same cytokines are released in asthmatic inflammation. NOS2 may be expressed in other cell types such as alveolar macrophages, eosinophils, and other inflammatory cells (26). Further evidence that the increase in exhaled NO is derived from increased NOS2 expression is the observation that corticosteroids inhibit inflammatory induction of NOS2 in epithelial cells (22, 27), decrease expression in bronchial biopsies of asthmatic patients (26), and also reduce exhaled NO concentrations in asthmatic patients (28) (Figure 1, *panel B*).

Nonenzymatic sources of NO. NOS is not the only source of NO in exhaled air, and exhaled NO is not therefore a direct measure of NOS activity in the lower respiratory tract. NO reacts with thiol-containing molecules such as cysteine and glutathione to form *S*-nitrosoproteins and *S*-nitrosothiols (29). Approximately 70 to 90% of NO is released by *S*-nitrosothiols, which therefore provide a major source of NO in tissues (30). *S*-nitrosothiols are potent relaxants of human airways and may play an important role in sequestration, releasing, and transportation of NO to its site of action (29).

NO in exhaled air may also be derived from nitrite protonation to form nitrous acid, which releases NO gas with acidification (31). This pH-related pathway has been implicated in acute asthma, when pH in expired condensate is low (32).

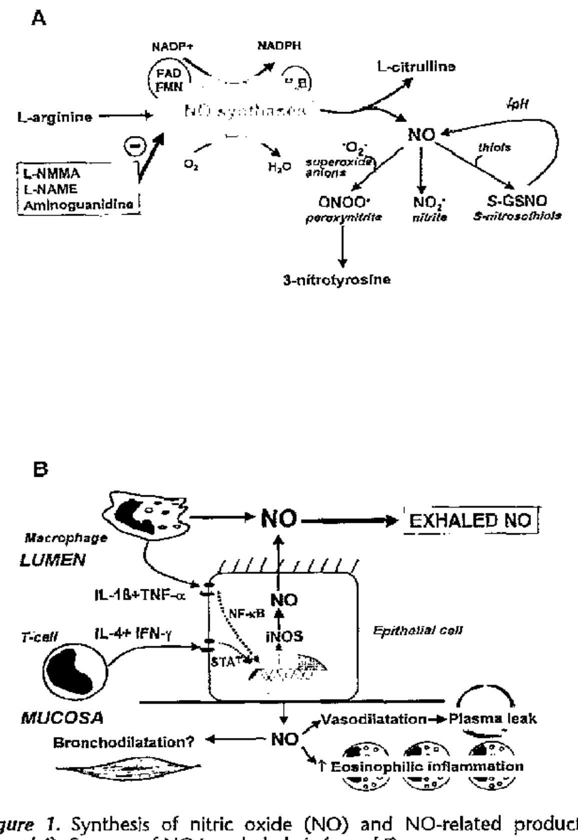

Figure 1. Synthesis of nitric oxide (NO) and NO-related products (*panel A*). Sources of NO in exhaled air (*panel B*).

Anatomic origin. NO is produced along the entire length of human airways. The conducting airways secrete NO into the lumen, which mixes with alveoli NO during exhalation, resulting in the observed expiratory concentration. The levels of NO derived from the upper respiratory tract (200 to 1,000 ppb) (33–35) and sinuses (1,000 to 30,000 ppb) (36) are a hundredfold higher than exhaled NO measured in the lower respiratory tract (1 to 9 ppb) (33, 34, 37–42). Several factors may contribute to high nasal levels. The paranasal sinuses produce a high level of NO (43). There is a dense innervation with NOS1-immunoreactive nerve fibers around nasal blood vessels (44). Vasculature-derived NO, however, is not the major source of NO in nasal mucosa, as neuropeptide Y, a powerful vasoconstrictor, reduces nasal blood flow by 37%, but NO by only 7% (45). There appears to be constitutive expression of NOS2 (46) and the transcription factor NF-κB in nasal mucosa (47). Interestingly, the NO outputs from the nostrils are significantly lower on the operated side (site with the reduced NO-generating surface) in patients who have undergone unilateral medial maxillectomy (48).

The source of NO in the lower respiratory tract is also of mixed origin and may be derived from airway and alveolar epithelial cells, which express both NOS3 and NOS1. The contribution of endothelial-derived NO is minimal, as inhaled NOS inhibitors are able to reduce exhaled NO by 40 to 70% (49–51) without any effect on the systemic circulation. By contrast, L-NMMA infusion modulates blood pressure and heart rate but has only a minimal effect on exhaled NO (49).

Simultaneous measurement of expired CO_2 and NO demonstrate that exhaled NO precedes the peak value of CO_2 (end-tidal), suggesting that NO is derived from airways rather than from alveoli (33, 52). Direct sampling via fiberoptic bronchoscopy in normal subjects shows a similar levels of NO in trachea and main bronchi to that recorded at the mouth, thus indicating that there is NO derived from the lower airways (33, 42). Exhaled NO is therefore most likely to be of epithelial rather than of endothelial origin, and most NO is derived from airways rather than from alveoli.

Measurement

Expiratory flow, soft palate closure, and dead space air may all influence exhaled NO levels. Therefore, exhaled NO is usually determined during single-breath exhalations against a resistance (38) (Figure 2, *panel A*) (28, 40, 53) to prevent contamination with nasal NO (54, 55), or using reservoir collection with discarding of the dead space (56). However, this method has proven difficult for some children, who may have trouble maintaining a constant flow, and recently a simple flow-driven method for online NO measurements has been developed that does not require active patient cooperation (57). Recently, single breath analysis of exhaled NO has been successfully performed in the newborn when exhaled air was sampled from the tip of a thin nasal catheter placed in the hypopharynx (58). The most commonly used method to measure nasal NO is to sample nasal air directly from one nostril using the intrinsic flow of the chemiluminescence analyzer (36). A novel method of measuring exhaled NO at several exhalation flow rates has recently been described that can be used to approximate alveolar and airway NO production (59). NO is continuously formed in the airways. Mixing during exhalation between the NO produced by the alveoli and the conducting airways, explains its flow dependency (55) and accumulation during a breathhold (33). A relatively simple and robust two-compartment model of NO has been developed that is capable of simulating many important features of NO exchange in the lungs (60). The model assumes that the lung consists of two well-defined, separate regions: a rigid airway compartment and a well-mixed, expansile alveolar compartment. Both compartments seem to contribute to exhaled NO, and the relative contributions of each seems to be a function of minute ventilation (60). Finally, the model suggests that the relationship between exhaled NO at end-exhalation may be a simple, effective, and reproducible technique for determining the relative contribution of the airways and alveoli to exhaled NO.

It is therefore important to register the flow rate if NO is expressed as a concentration The flow rate recommended in 1997 by a Task Force of the European Respiratory Society is 10 to 15 L/min or 167 to 250 ml/s (53). Most investigators have used about 100 ml/s, but a more recent recommendation from the American Thoracic Society suggests 50 ml/s (61).

Factors Affecting Exhaled NO Measurements

Exhaled and nasal NO in healthy subjects is independent of age, sex, and lung function (34, 62). There is no evidence for significant diurnal variation (63), and exhaled NO measurements are highly reproducible in normal subjects (64, 65). Different phases of the menstrual cycle may influence exhaled NO (66), as estrogen activates NOS3 in airway epithelial cells (67).

There are several major factors, which may change NO levels in normal subjects (Table 1). Either intravenous, or inhaled, or digested L-arginine, the substrate for NOS, increase exhaled NO levels in normal subjects (68–70). Conversely nebulized L-NMMA and L-NAME, nonspecific inhibitors of NOS, reduce exhaled NO (28, 50) and nasal NO (71, 72). Some routinely used tests can transiently reduce exhaled NO; for example, repeated spirometry (73, 74), physical exercise (75), sputum induction (76). Environmental factors such as NO ozone and chlorine dioxide are known to increase exhaled NO levels (77–79). Habitual factors such as smoking (80, 81) and alcohol

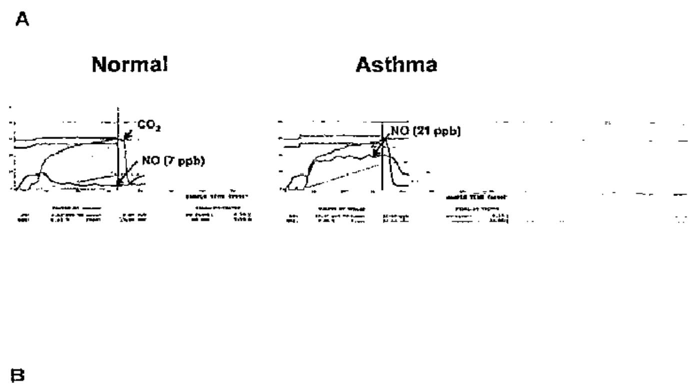

Figure 2. Traces of exhaled NO in normal subject and in patient with asthma (*panel A*). Scatterogram of exhaled and nasal NO in normal and in asthmatic subjects (*panel B*). Reference 33.

TABLE 1. FACTORS AFFECTING EXHALED AND NASAL NO MEASUREMENTS IN HEALTHY SUBJECTS

Increased NO	Decreased NO
Pharmacologic	
Papaverin (71)	Oxymetazoline (71, 72)
Sodium nitroprusside (458)	NOS inhibitors (50, 51, 71, 72)
L-arginine (68, 186)	
ACE inhibitors (enalapril) (216)	
Physiologic and procedural	
Arginine ingestion, nitrite/ nitrate-enriched food (70)	Repeated spirometry (73, 74)
	Acute and transient after forced exhalation (74)
	Physical exercise (75)
	Menstrual cycle (66)
	Sputum induction (76)
	Body temperature reduction (459)
Environmental, occupational	
Air pollution (NO, ozone) (77)	Water vapour, CO_2, nitrous oxide, heptane (462)
Occupational hazards:	100% inspired O_2 (463)
Fluoride, dust (221)	Moderate altitude (464)
Ozone, chlorine dioxide (78)	
Rubber latex (222)	
Formaldehyde (domestic) exposure (460)	
Electromagnetic field generated by cellular phone (nasal NO) (461)	
Habitual	
	Smoking (80, 81)
	Alcohol ingestion (82, 83)
Infections	
URTI (84–86)	

Definition of abbreviations: ACE = angiotensin-converting enzyme; URTI = upper respiratory tract infection.

ingestion (82, 83) reduce exhaled NO. Upper respiratory infection significantly increases exhaled NO (84, 85) and nasal NO (86).

Asthma

Increased levels of exhaled NO have been widely documented in patients with asthma (Figure 2, *panel B*) (28, 87). The increased levels of exhaled NO in asthma have a predominant lower airway origin (33, 42) and are most likely due to activation of NOS2 in airway epithelial and inflammatory cells (21, 26). However, there may be a small contribution from NOS1 as polymorphisms of NOS1 gene are correlated with exhaled NO (13). Exhaled NO may be further elevated by NO substrate L-arginine (69).

Diagnosis and epidemiology. An elevation of exhaled NO is not specific for asthma, but an increased level may be useful in differentiating asthma from other causes of chronic cough (88). The diagnostic value of exhaled NO measurements to differentiate between healthy subjects with or without respiratory symptoms and patients with confirmed asthma has been recently analyzed by Dupont and colleagues (89) with 90% specificity and 95% positive predictive value when exhaled NO > 15 ppb is used as a cutoff for asthma. The intraindividual coefficient of variation (CoV) of exhaled NO in normal subjects was 15.8% within an interval of 7 d, and 16.8% within 23 d, suggesting that the change of exhaled NO by 30 to 35% or more within the interval of 1 to 3 wk would be abnormal (62). Exhaled and nasal NO may be used to identify subjects with atopy, because nonatopic asthmatics have normal exhaled NO (90). There is a strong association between elevated exhaled and nasal NO and skin prick test scores, total IgE (91), and blood eosinophilia (92) in mild asthma. Elevated nasal NO is also related to the size of skin test reactivity in asymptomatic asthmatic subjects (93). This may denote "subclinical" airway inflammation.

Another potential use of exhaled NO levels in patient management is the prediction of future asthma. An elevated exhaled NO may be found in patients with "subclinical" forms of asthma (normal lung function, negative bronchodilator tests, and elevated sputum eosinophilic cationic protein concentrations) (94, 95). Elevated levels of NO in patients with "subclinical asthma" are not in conflict with the specificity of exhaled NO as a marker to diagnose asthma, as lack of current asthma symptoms does not exclude the diagnosis of asthma. Perhaps, this subclinical airway inflammation, which is reflected by elevated levels of exhaled NO in adolescent asymptomatic patients with asthma remission (96), should be treated with corticosteroids to prevent the risk of becoming clinically manifest again. This category of patients with "subclinical" forms of asthma, especially children, may be predisposed to develop asthma in the future (97). This may be studied in epidemiologic studies, in which the reservoir collection of exhaled NO has proved to be useful (98, 99). Airway responsiveness measurements (PC_{20}) in this "high risk" group make the combination of exhaled NO and PC_{20} a more specific test for allergic asthma. This has recently been demonstrated in a study of more than 8,000 adolescents in Norway (100). Because of the noninvasive character and practicality of exhaled and nasal NO measurements they may be used cost effectively for screening of large populations.

Atopy and exposure to proinflammatory stimuli. Exhaled NO is elevated in allergic/atopic adults and children (97, 101, 102). It is further increased as a result of allergen exposure such as during the late phase response to allergen challenge (103, 104), during the grass pollen season (105), or during exposure to indoor allergens (106, 107). In subjects sensitive to house dust mites (HDM) the wheal size for HDM correlates with exhaled and nasal NO levels (93). Both adults (97) and children (102) with atopic asthma have higher levels of exhaled NO than do patients with nonatopic asthma, even without airway hyperresponsiveness (108).

Exhaled NO may represent a useful biomarker of individual exposure to air pollutants, as even healthy subjects may have elevated exhaled NO levels on days with high outdoor air pollution (79, 109). This may reflect an airway inflammatory response to ozone and nitrogen dioxide (110).

Asthma monitoring. It is difficult to monitor the response of different classes of anti-inflammatory drugs in asthma, as there is no single test that can be used to quantify airway inflammation. Peripheral blood markers are unlikely to be adequate as the most important mediator and cellular responses occur locally within airways. Eosinophils in induced sputum originate from more proximal rather than small airway (111). It is clear that different markers of airway inflammation should be considered together to monitor asthma (3).

Exhaled NO has been used to monitor the effect of anti-inflammatory treatment in asthma (6, 112) and asthma exacerbations, both spontaneous (40) and induced by steroid reduction (113, 114). There is a lack of long-term serial studies of exhaled NO, together with other markers of airway inflammation in sputum and exhaled condensate, lung function and symptoms. Exhaled NO behaves as a "rapid response" marker, which is extremely sensitive to steroid treatment, as it may be significantly reduced even after 6 h following a single treatment with a nebulized corticosteroid steroid (115), or within 2 to 3 d after inhaled corticosteroids (112), reaching maximal effect after 2 to 4 wk of treatment (112, 113, 116–120).

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An important issue in asthma management is to prevent overtreatment of patients with steroids. The high sensitivity of exhaled NO to corticosteroid treatment is an advantage, as higher doses of inhaled steroids are not necessary to improve asthma control, e.g., in mild persistent asthma (3). We have demonstrated a dose-dependent reduction in exhaled NO and improvement in asthma symptoms in patients with mild asthmatics after treatment with low doses of inhaled corticosteroids (120), whereas the reduction in sputum eosinophils and similar improvement in symptoms was observed only after the higher dose of steroids (117). This suggests that exhaled NO levels may be too sensitive to determine whether inflammation is adequately controlled (3).

Although exhaled NO levels are normal in patients with moderate asthma treated with corticosteroids (28), increased levels have been observed in patients with severe asthma, despite treatment with oral corticosteroids (98, 121). Individual NO values such as individual peak expiratory flows should be established and monitored, and when the levels are above or below a certain reference level, steroid treatment should be either reduced or increased.

A considerable advantage of exhaled NO is that NO levels may increase before any significant changes in other parameters such as lung function and sputum eosinophils and may therefore serve as an early warning of loss of control (4). Thus, exhaled NO levels increase by 40 and 100% after 2 and 4 wk, respectively, after the reduction in steroid treatment (114). This increase in exhaled NO levels is accompanied by lung function deterioration and asthma symptoms. Although the baseline high number of eosinophils in sputum of patients who eventually develop exacerbations is a good predictor of asthma deterioration, the changes in eosinophils after the steroid reduction are slow (114). Prospective studies, which look at asthma outcomes over a prolonged period of time, where NO is used as a decision point for modifying inhaled corticosteroid treatment will be needed to evaluate the value of exhaled NO as a useful way of monitoring asthma.

Disease severity and control. Treatment with inhaled corticosteroids reduces exhaled NO levels, and therefore exhaled NO cannot be directly related to asthma severity.

Exhaled NO levels are almost three times higher in children with recent symptoms than in symptom-free subjects (122), and are further elevated during the asthma attack in both adults (123) and children (124, 125). In fact, the levels of NO in children with acute severe asthma (125) are more than 2-fold higher than in children with less severe wheezing exacerbations and almost 4-fold higher than in children with first-time wheeze (124). A reduction in exhaled NO (by 65% after 5 d of corticosteroid therapy) is accompanied by clinical and FEV_1 improvement from asthma exacerbations in children (126), and NO has been a more sensitive marker of asthma activity than serum ECP or soluble interleukin-2 receptors (127). Higher exhaled NO levels are related to asthma symptoms and β_2-agonist use in patients with difficult severe asthma (98). Exhaled NO is increased in patients who remain symptomatic despite oral steroids and who have a relative steroid resistance, and may therefore be useful to quantify steroid resistance in asthma.

It is most likely that exhaled NO is related to asthma control rather than to asthma severity (3), and that serial NO measurements in individual patients over time may be useful to identify patients requiring changes in therapy. In a recent study, Sippel and coworkers (128) have shown that exhaled NO was significantly correlated with markers of asthma control such as asthma symptoms within the previous 2 wk, dyspnea score, daily use of rescue medication, and reversibility of airflow obstruction. However, exhaled NO levels were not correlated with the following markers of asthma severity: history of respiratory failure, health care use, or fixed airflow obstruction.

It is reasonable to believe that subclinical airway inflammation, which is reflected by elevated levels of exhaled NO in adolescent asymptomatic patients with asthma remission (96), should be treated with corticosteroids to prevent this continuous risk of becoming clinically manifest again. However, only longitudinal studies can answer the question whether exhaled NO and bronchial hyperresponsiveness, for example, each reflecting different aspects of the inflammatory process, may guide the anti-inflammatory treatment to prevent asthma relapse later in life.

Although research in asthma has concentrated on complex proinflammatory mechanisms, it is likely that defective expression of cytokines that inhibit allergic inflammation such as interleukin 10 (IL-10), interleukin 12 (IL-12), and interferon gamma might also be important, particularly in determining disease severity and persistence of inflammation in the airways (129). Therapy based on these cytokines might also be useful, with the advantage that it restores the balance of endogenous cytokines. Recently, it has been shown that adenovirus-mediated human IL-10 gene transfer *in vivo* into lung isografts ameliorates subsequent ischemia-reperfusion injury and results in reduced neutrophil sequestration, and downregulation of iNOS mRNA expression (130). Potentially, exhaled NO may be useful to monitor this type of treatment.

Relationship to other markers of asthma. The traditional means of monitoring asthma have limitations. Lung function and PC_{20}, measurements are not directly related to airway inflammation, have little room for improvement in mild asthma (FEV_1), and are affected by bronchodilators. Both parameters are slow to change and are not able to distinguish the effect of different doses of steroids. There are several areas in which exhaled NO measurements may be advantageous over the traditional means of asthma monitoring: screening for atopy, monitoring the impact of hazardous environmental factors, identification and monitoring of asthma exacerbations, and assessment of the adequacy of anti-inflammatory treatment.

Exhaled NO in patients with asthma is correlated with sputum eosinophils (117, 131, 132) and methacholine reactivity (133, 134), as well as peak flow variability (113, 116). However, the relationship between exhaled NO and airway inflammation is still uncertain, and in smaller studies no significant relationship is seen between exhaled NO and eosinophils in bronchial biopsies or bronchoalveolar lavage (116), and the induction of sputum eosinophils by inhaled LTE_4 is not associated with increased exhaled NO (135, 136). This may indicate that increased exhaled NO reflects some, but not all, aspects of airway inflammation, and further work is needed to determine how it relates to some other markers of airway inflammation. On the other hand, a more comprehensive spectrum of inflammatory markers (for example, IL-4, IL-5, IL-6, IL-8, IL-10, and TNF-α) can be measured in induced sputum, and in the future these should be correlated with changes in exhaled NO.

Corticosteroids. Systemic corticosteroids have no effect on exhaled NO in normal subjects, but they decease its levels in patients with asthma (40, 50). Oral dexamethasone (4 mg/d for 2 d) similarly has no effect on exhaled NO or on serum concentrations of interferon-γ and IL-1β in normal subjects (137).

A large dose (1 mg/kg/d for 5 d) of oral prednisolone normalized exhaled NO in infants and young children with wheezing exacerbations (124), whereas the same dose in children with more severe asthma only shifted their exhaled NO down to the levels of mild-to-moderate asthma, in spite of the improvement in lung function (125). A cumulative dose of methylprednisolone (180 to 500 mg) causes 36% reduction

within 50 h in the majority of severe adult patients with severe, acute asthma (40), and a combination of oral prednisolone and inhaled steroids reduces exhaled NO by 65% in children with acute asthma (126).

Recently, it has been shown that NO levels correlate with the percentage improvement in FEV_1 from baseline to the poststeroid (30 mg prednisolone/d for 14 d) postbronchodilator value. A NO level of > 10 ppb at baseline has a positive predictive value of 83% for an improvement in FEV_1 of $\geq$ 15%, and therefore may be useful in predicting the response to a trial of oral steroid in asthma (138).

A key question is why has it been so difficult to show a dose-dependent effect of inhaled corticosteroids in the treatment of asthma? First, it is possible that the small change in doses makes it difficult to detect changes in asthma symptoms and lung function (FEV_1). Secondly, the currently recommended doses may be at the upper end of the dose-response curve, making it difficult to detect a relatively small change in dose. In view of concerns about systemic effects and the better effects of adding an inhaled long-acting β_2-agonist compared with doubling the dose of inhaled steroid, there is now a trend towards use of lower doses of inhaled corticosteroids. Exhaled NO as an inflammatory marker sensitive to corticosteroids may be the ideal tool to demonstrate a dose-response effect and to adjust the dose in clinical practice. It may also be useful in patients using a fixed combination inhalers (corticosteroids and long acting β_2-agonist) to ensure that inflammation is controlled, as this may be difficult to assess from symptoms when a long-acting bronchodilator is taken. On the other hand, caution should be exercised as once-daily combination therapy

In fact, inhaled corticosteroids reduce exhaled NO in asthmatic patients (112) and this effect is dose-related (117). However, a plateau effect on exhaled NO measured after 6 to 12 h since the last treatment may be seen at a dose of 400 μg budesonide and higher (117, 139) in contrast to dose-related improvements in adenosine monophosphate and methacholine reactivity up to 1,600 μg in patients with mild-to-moderate asthma (120, 140). The effect of inhaled steroids on exhaled NO is very rapid and may occur within 6 h after a single high-dose (8 mg) of budesonide (Pulmicort Respules) in symptomatic moderate asthma (115). Therefore, chronic and acute reduction in exhaled NO may be of a different magnitude. Recently, it has been shown that the onset of action of inhaled BUD on exhaled NO and the time to reach the maximal reduction were also dose-dependent (120). A gradual reduction in exhaled NO is seen during the first week of regular treatment (112, 119, 120) with maximal effect between 3 wk (112, 118) or 4 wk (116, 117).

It is still uncertain whether exhaled NO is useful to direct changes in asthma therapy. Recently, it has been shown that exhaled NO values above 13 ppb had a sensitivity of 0.67 and a specificity of 0.65 to predict a step up in therapy (141), but clearly more studies are needed using exhaled NO to direct therapy.

Corticosteroids may reduce exhaled NO by directly inhibiting the induction of NOS2 (22) or by suppressing the proinflammatory cytokines that induce NOS2. There is inhibition of NOS2 immunoreactivity with inhaled corticosteroid treatment in asthmatic patients and a parallel reduction in immunoreactivity for nitrotyrosine, which may reflect local production of peroxynitrite from an interaction of NO and superoxide anions (26).

β_2-agonists. Neither short-acting (112, 125, 142–145) nor long-acting (125, 139, 142, 144, 146) β_2-agonists reduce exhaled NO. This is consistent with the fact that they do not have any anti-inflammatory effects in asthma, although it has been shown that regular treatment with inhaled formoterol reduces inflammatory cells in the mucosa of asthmatic patients (147). There may even be a short-term increase in exhaled NO after β_2-agonists, which may be due to opening up of airways with higher local NO concentrations (148).

Antileukotrienes. The leukotriene receptor antagonist pranlukast blocks the increase in exhaled NO when inhaled corticosteroids are withdrawn (149), and montelukast rapidly reduces exhaled NO by 15 to 30% in children with asthma (150). Antileukotrienes have a moderate effect in patients with asthma and seasonal allergic rhinitis (151, 152). Both formoterol and zafirlukast were equally effective in maintaining asthma control, and zafirlukast caused a significant reduction in exhaled NO (143).

NOS inhibitors. Nebulized L-NMMA and L-NAME, which are nonselective inhibitors of NOS, both reduce exhaled NO in asthmatic patients, although this is not accompanied by any changes in lung function (50, 153). Aminoguanidine, a more selective inhibitor of NOS2, reduces exhaled NO in asthmatic patients, but it has little effect in normal subjects, indicating that NOS2 is an important source of the increased exhaled NO in asthma (51).

Prostaglandins. Prostaglandin $(PG)E_2$ down-regulates NOS2 expression (154) and inhaled PGE_2 and $PGF_{2\alpha}$ decrease exhaled NO in normal and in asthmatic subjects (155).

Other drugs. The immunosuppressive drugs cyclosporin and rapamycin inhibit NOS2 expression (156), suggesting that exhaled NO can be used to monitor their effect. Ibuprofen, a cyclooxygenase inhibitor, reduces the elevated levels of exhaled NO in normal subjects after intravenous administration of endotoxin (157), and indomethacin partially prevents an increase in exhaled NO and asthma symptoms in patients whose dose of steroids was reduced (158). A low dose of theophylline has no effect on exhaled NO levels in asthmatic patients (159). Nebulized IL-4 receptor (altrakincept) reduces exhaled NO in patients with moderate asthma (160).

COPD

Exhaled NO levels in patients with stable COPD (80, 81, 161) and chronic bronchitis (162) are lower than in either smoking or nonsmoking asthmatics (163) and are not different from those in normal subjects. This reduction in exhaled NO is due to the effect of tobacco smoking, which down-regulates eNOS (164) and reduces exhaled NO (80), suggesting that this may contribute to the high risk of pulmonary and cardiovascular disease in cigarette smokers. In addition to the effects of cigarette smoking, a relatively low value of exhaled NO in COPD may reflect more peripheral inflammation than in asthma, low NOS2 expression (161), and increased oxidative stress that may consume NO in the formation of peroxynitrite (165).

Patients with unstable COPD, however, have high NO levels compared with stable smokers or ex-smokers with COPD (166), which may be explained by increased neutrophilic inflammation and oxidant/antioxidant imbalance. Eosinophils that are capable of expressing NOS2 and producing NO are present in exacerbations of COPD (167). Acidosis, which is frequently associated with exacerbations of COPD, may increase the release of NO (32). Pulmonary hypertension has the opposite effect, as COPD patients with cor pulmonale have low exhaled NO levels (168), which may reflect their impaired endothelial NO release.

A small proportion of patients with COPD appear to response to corticosteroids, and these patients, who are likely to have coexistent asthma, have an increased proportion of eosinophils in induced sputum (169). These patients also have an increased in exhaled NO (170). This suggests that exhaled NO may be useful in predicting which patients with COPD will respond to long-term inhaled corticosteroid treatment.

Cystic Fibrosis

Surprisingly, exhaled and nasal NO levels are significantly lower in patients with cystic fibrosis (CF) than in normal subjects, despite the intense neutrophilic inflammation in the airways (35) (Figure 3) (171) leading to the release of superoxide anions, which convert NO to nitrate and may result in the formation of peroxynitrite (172). Increased oxidative stress in CF is likely to be a consequence of this neutrophilic inflammation, malnutrition, and IL-10 deficiency (173, 174). Although there is a trend toward both exhaled and nasal NO being higher in patients who were not homozygous for the ΔF508 CF transmembrane regulator mutation (175, 176), there is no strong association between exhaled NO and disease severity in CF (176) or infection with *Pseudomonas* (35).

There are several possible reasons for the low levels of NO in patients with CF. First, there is a deficiency of NOS2 in patients with CF (177). Constitutive expression of NOS2, which has been demonstrated in normal human airway epithelium, and of non-CF mouse is essentially absent in the epithelium of CF airways (178). Neutrophils enhance expression of NOS2 in normal human bronchial epithelial cells but not in CF epithelial cells (179). The low expression of NOS2 would account for the low levels of NO in nasal as well as exhaled air. Secondly, an association between the length of a repeat polymorphism in the NOS1 gene and exhaled NO in patients with CF has recently been demonstrated, and exhaled NO is significantly lower in patients with CF with two alleles with a high number of repeats than in those alleles with fewer repeats at this locus (180). Interestingly, *Pseudomonas aeruginosa* colonization is more common in patients with CF with high numbers of repeats in the NOS1 gene, hence with lower exhaled NO.

Sexual hormones have impact on cystic fibrosis transmembrane mRNA expression (181) and it is not unusual that female patients with CF have reported worsening of lung symptoms prior to menstruation. Changes in exhaled NO during the menstrual cycle with the lowest NO levels during menstruation have been observed (66). Although NO is a weak bronchodilator and the physiologic significance of this finding is still not known, it has been shown that FEV_1 was significantly lower during menstruation in female patients with CF (182).

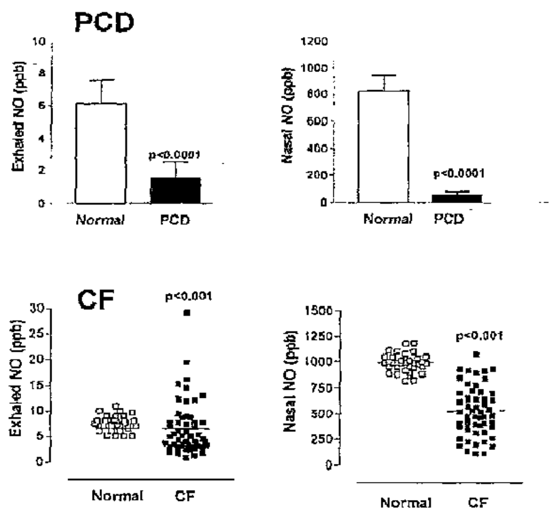

Figure 3. Exhaled and nasal NO in primary ciliary dyskinesia (PCD) (from Reference 186) and cystic fibrosis (CF) (from Reference 35).

Bronchiectasis

An increase in exhaled NO is found in bronchiectasis and the increase in NO is related to the extent of disease as measured by a computerized tomography score (183). As in asthma, the elevation of exhaled NO is not seen in patients treated with inhaled corticosteroids. This suggests that exhaled NO in bronchiectasis may reflect active inflammation in the lower airways and may be used to monitor disease activity. This is supported by increased NOS2 expression in lungs from patients with bronchiectasis (184). However, in another study, exhaled NO levels were not elevated compared with normal subjects in clinically stable patients with bronchiectasis, and it was suggested that NO is either trapped in viscous airway secretions or removed by reaction with reactive oxygen species (185).

Primary Ciliary Dyskinesia

Primary ciliary dyskinesia (PCD), including Kartagener's syndrome, is a genetic disease characterized by defective motility of cilia, in which the levels of exhaled NO are very low compared with normal subjects (186) (Figure 3) (187, 188). Such low values of exhaled and nasal NO are not seen in any other condition and are therefore of diagnostic value. Measurement of exhaled NO might be used as a screening procedure to detect PCD among patients with recurrent chest infections or male infertility caused by immotile spermatozoa, and the diagnosis of PCD is then confirmed by the saccharine test, nasal nitric oxide, ciliary beat frequency, and electron microscopy (189). NO plays an important role in bactericidal activity in the lungs, sodium and chloride transport in nasal epithelium, and ciliary beating (190), so that a lack of endogenous NO production might contribute to the characteristic recurrent chest infections in patients with PCD. Low levels of exhaled and nasal NO in patients with PCD are related to mucociliary dysfunction (186, 191), and treatment with NO donor L-arginine increases nasal NO and also improves mucociliary transport in patients with PCD (3, 186). The mechanism for such a low NO production by nasal and airway epithelia in PCD is unknown, but it might be linked to genetic abnormalities in NOS2 gene expression as in CF.

Rhinitis

The levels of NO derived from the upper respiratory tract are more than 100-fold higher than those from lower airways. This fact is mostly due to its high production in human paranasal sinuses (43), which is due to high basal activity of constitutively expressed form of NOS2 (192), and nasal NO may be significantly reduced by L-NAME in normal subjects (72), and this inhibition of NOS may induce hyperresponsiveness of the nasal airway (193). Strong NOS3- and weak NOS2-immunoreactivity are found in nasal epithelium and submucosal glands of normal subjects, but NOS2 reactivity is increased in patients with allergic rhinitis (46). There is increased immunoreactivity of nitrotyrosine in the nasal mucosa of patients with perennial rhinitis and is related to the severity of the nasal symptoms (194). However, an increased expression of iNOS is not necessarily associated with a higher 3-nitrotyrosine-labeling intensity (195), suggesting that iNOS-derived NO may have a role in the pathophysiology of rhinitis, but the production of peroxynitrite in patients with rhinitis is not dependent on the level of iNOS alone. Eotaxin causes chemotaxis of eosinophils, an increase of nitrotyrosine-immunoreactivity in nasal mucosa and increased levels of nasal NO in clinically symptomatic patients with allergic rhinitis (196). Instillation of LTB_4 into the nasal

segment caused a time-dependent increase in the volume of airway fluid and in the recruitment of neutrophils in dogs, and was prevented by L-NAME (197). Recently, it has been shown that the nasal decongestants oxymetazoline and xylometazoline, frequently used in the topical treatment of rhinitis and sinusitis, may have a dose dependent inhibitory effect on total iNOS activity (198).

Elevated nasal NO has been reported in allergic and perennial rhinitis (199, 200), which is reduced by treatment with nasal corticosteroids (200). Similar results are seen in children with allergic rhinitis (201). In addition, exhaled NO is also significantly elevated in allergic rhinitis in the nonpollen season and is increased further in the pollen season (202). However, the differences between the levels of nasal NO in rhinitis compared with those in normal subjects and much less marked than the differences between exhaled NO between patients with asthma and normal subjects because of the very high baseline values. This makes nasal NO less useful for diagnosis and monitoring treatment in rhinitis than exhaled NO in asthma.

Interstitial Lung Diseases

Systemic sclerosis. In patients with systemic sclerosis who have developed pulmonary hypertension, there is a reduction in exhaled NO compared with that in normal subjects and with that in patients with interstitial lung disease without pulmonary hypertension (203, 204). This may be due to reduced expression of NOS3 in pulmonary vessels, or a reduction in the pulmonary vascular endothelial surface. However, the presence of NOS3 in pulmonary vessels is variable, and it has been found to be either reduced (205–207), increased (208), variable (209), or unaltered (210).

Fibrosing alveolitis. There is strong expression of nitrotyrosine and NOS2 in macrophages, neutrophils, and alveolar epithelium in lungs of patients with idiopathic pulmonary fibrosis with active inflammation during the early to intermediate stage of the disease (211). This is consistent with elevated levels of exhaled NO in patients with fibrosing alveolitis. Increased exhaled NO levels are associated with disease activity, as assessed by BAL lymphocyte counts, and are reduced in patients treated with corticosteroids (212).

Sarcoidosis. Cytokines, including TNF-α and interferon-γ, are increased in the pulmonary inflammation of sarcoidosis and there is an up-regulation of NOS2 in respiratory epithelium and granulomata in patients with sarcoidosis (213). The magnitude of the rise in exhaled NO in sarcoidosis may be related to the activity of the disease and is reduced by steroid therapy. This is, perhaps, the reason behind two conflicting observations reporting either elevated (213) or normal (214) exhaled NO in patients with active pulmonary sarcoidosis.

Pulmonary Hypertension

The pathogenesis of pulmonary hypertension remains poorly understood. Vasoconstriction is likely to be a major factor in the initial stages of the disease, and a reduction in endogenous NO may contribute to the development of pulmonary hypertension. In fact, nebulized epoprostenol increased exhaled NO in patients with pulmonary hypertension, but not in normal control subjects, suggesting that this effect on the hypertensive circulation has a NO-related mechanism (215). In contrast, the angiotensin-converting enzyme (ACE) inhibitor enalapril, used to treat pulmonary hypertension, increases exhaled NO levels in normotensive subjects, but not in patients with systemic hypertension (216).

Biochemical reaction products of NO are inversely correlated with pulmonary artery pressures in patients with primary pulmonary hypertension and with years since the diagnosis (217). This may reflect reduced expression of NOS3 in patients with pulmonary hypertension, as reduced NOS3 expression has been reported in patients with primary pulmonary hypertension (205–207). In fact, aerosolized NOS2 gene transfer increases pulmonary NO production and reduces hypoxic pulmonary hypertension in rats (218) and may be a promising future strategy to target pulmonary vascular disorders.

However, interpretation of these low NO levels should be made cautiously and in the context of potential influence of Hb on NO. Although stimulation of NO production by pulmonary vascular endothelial cells in response to shear stress has been described, it is not an important determinant of NO production. Low exhaled NO in patients with pulmonary hypertension may be consistent with flow redistribution from alveolar septal capillaries to extra-alveolar vessels and decreased surface area or a direct, stretch-mediated depression of lung epithelial NO production (219), or increased Hb NO scavenging. It may be difficult to use exhaled NO changes as an accurate measure of lung tissue NO production.

Occupational Diseases

Allergens from rats, mice, guinea pigs, or rabbits cause as much as 30% of exposed persons to develop specific immunoglobulin E (IgE) responses. Laboratory animal allergy (LAA) is among the highest occupational risks for asthma. Exhaled NO is raised in subjects with LAA symptoms and correlates with symptom severity (97). The progressive increase in exhaled NO from asymptomatic to early LAA to symptomatic asthma suggests that exhaled NO measurements may be useful in monitoring occupational asthmas, and of environmental health effects of air pollution (220) in epidemiologic surveys. Recently, measurement of exhaled NO and induced sputum were evaluated in occupational asthma. Aluminum potroom workers (exposure to dust and fluorides) with asthmalike symptoms had higher concentrations of exhaled NO than did those with no symptoms (221), suggesting that exhaled NO may be an early marker of airway inflammation in potroom workers. High levels of exhaled NO and asthmalike symptoms in subjects with occupational exposure to high levels of ozone and chlorine dioxide (78), or in swine confinement workers (162), may indicate the presence of chronic airway inflammation. Latex sensitivity is an increasing problem among healthcare workers. Although allergen challenge with natural rubber latex increased exhaled NO levels after 22 h in some subjects with suspected occupational asthma (222), further studies are needed to demonstrate a clear relationship between exhaled NO and routine latex workplace exposure.

Infections

NO may play an important role in nonspecific host defenses against bacterial, viral and fungal infections. One of the general mechanisms of antimicrobial defenses involving NO is *S*-nitrosylation by NO of cysteine proteases, which are critical for virulence, or replication of many viruses, bacteria, and parasites. The reduced endogenous NO production, resulting in low exhaled and nasal NO levels, may contribute to recurrent chest infections in patients with PCD or CF, as discussed above. Low nasal NO is associated with colonization of the upper respiratory tract with *Staphylococcus aureus* in active Wegener's granulomatosis (223).

Viral infections. Exhaled, but nasal, NO is elevated during viral infections in adults and in children (84, 86). Exhaled NO is also increased in experimental human influenza (224) and rhinovirus infection (225). The increase in NO production during viral infection is likely to be protective, as NO inhibits

virus replication either by inhibiting viral RNA synthesis, or/and by S-nitrosylation of the cysteine proteases that are critical for virulence and replication of viruses (226). Viral infection may also induce the expression of NOS2 via activation of NF-κB and other transcription factors (227). Exhaled (228) and nasal NO (229) in HIV positive patients is less than in control subjects, and NO synthesis is further depressed in terminally ill patients with HIV (230), suggesting that low NO may indicate a mechanism of impaired host defense in HIV infection. This may be explained by an inhibitory role of the HIV type 1 regulatory protein Tat on NOS2 activity in a murine macrophage cell line (231).

Tuberculosis. NO plays an important role in resistance to *Mycobacterium tuberculosis* infection, and exposure of extracellular *M. tuberculosis* to < 100 ppm of NO for a short period (< 24 h) results in microbial killing (232). Elevated exhaled NO and NOS2 expression in alveolar macrophages is found in patients with active tuberculosis and is reduced with antituberculosis therapy (233).

Bacterial infections. Nitrate concentrations are significantly higher in BAL in immunosuppressed children with pneumonia than in normal control subjects (234), and elevated exhaled NO levels are found in patients with lower respiratory tract inflammation and chronic bronchitis (162).

Chronic Cough

Increased levels of exhaled NO do not accompany all forms of airway inflammation. Patients with chronic cough that is not attributable to asthma have lower NO values than do healthy volunteers and patients with asthma (88, 134), including those with cough caused by gastroesophageal reflux (235). Measurement of exhaled NO may therefore be a useful screening procedure for patients with chronic cough and would readily identify those patients with cough caused by asthma (88).

Lung Cancer

The levels of nitrite in epithelial lining fluid and exhaled NO are significantly higher in patients with lung cancer than in control subjects, and they are correlated with the intensity of NOS2 expression in alveolar macrophages (236). The level of nitrite was also significantly higher in epithelial lining fluid from patients with cancer, but the increased NO production is not specific to the tumor side and might be attributed to a tumor-associated nonspecific immunologic and inflammatory mechanism.

Lung Transplant Rejection

Monitoring endogenous NO release may be useful in lung transplantation. Loss of endogenous production of NO by cadaver lung allografts in the perioperative period (237), and the fact that reduced exhaled NO after hypoxia-reoxygenation might reflect bronchial epithelial dysfunction (238), may provide a rationale for interventions to restore NO production and, therefore, to improve the outcome of the surgery. The development of postlung transplant obliterative bronchiolitis is the commonest cause of late graft failure and is characterized by intense airway inflammation and high exhaled NO, which are higher than in either control subjects or stable lung transplant recipients (239). In stable lung transplant recipients, exhaled NO concentrations are highly dependent upon the severity of BAL neutrophilia and the intensity and extent of expression of NOS2 in the bronchial epithelium, but not in the subepithelial area (240). This suggests that serial exhaled NO measurements may have a role in the early detection of obliterative bronchiolitis (240) or of acute rejection (241).

Adult Respiratory Distress Syndrome

Adult respiratory distress syndrome (ARDS) is associated with a neutrophilic alveolar inflammation. In animal models of ARDS induced by endotoxin there is increased production of NO (242). Exhaled NO values are low, presumably because of the concomitant oxidative stress and consumption of NO by superoxide anions to form peroxynitrite (243). Association of reduced exhaled NO levels with the increases in pulmonary artery pressure and alveolar-arterial oxygen pressure and the decrease in lung compliance (244) suggests that exhaled NO may be an indicator of lung injury in adult patients after cardiopulmonary bypass.

Diffuse Panbronchiolitis

Diffuse panbronchiolitis (DPB), a pulmonary disease of unknown origin with chronic inflammation in the respiratory bronchioles leading to chronic chest infections resulting from mucociliary dysfunction, is the third disease (after primary ciliary dyskinesia, and cystic fibrosis) with diagnostically low nasal NO levels (245). Airway impaired NOS activity may be involved in its pathogenesis, and NO measurements may serve as a noninvasive test in the diagnosis of DPB.

CARBON MONOXIDE

Carbon monoxide (CO) is a gas that may be formed endogenously and is detectable in exhaled air.

Source of Exhaled CO

There are three major sources of CO in exhaled air: enzymatic degradation of heme, non–heme-related release (lipid peroxidation, xenobiotics, bacteria) and exogenous CO. The predominant endogenous source of CO (~ 85%) in the body is from the degradation of hemoglobin by the enzyme heme oxygenase (HO), and approximately 15% arises from degradation of myoglobin, catalase, NO synthases, guanylyl cyclase and cytochromes (246). Several bacteria produce CO (247), but this does not play an appreciable role in the turnover of CO that is inhaled or endogenously produced. Approximately 85% of the CO in the body is bound to hemoglobin in circulating erythrocytes and the remaining 15% is bound to other compounds (such as myoglobin) or in tissues, and less than 1% is unbound and dissolved in body fluid (248). Approximately 80% of the CO formed from heme degradation is exhaled (249). CO uptake or excretion across the skin is minimal, except in premature infants, and the amount of CO consumed by the tissues is very small (3% of the rate of endogenous CO production) (250).

There are several reasons to consider that the alveoli are the predominant site of exhaled CO in normal subjects. First, levels of exhaled CO measured at the end of exhalation are similar to those measured via a bronchoscope at the level of main bronchus (251). Second, exhaled CO levels are less flow- or breathhold-dependent than exhaled NO (252), suggesting less airway contribution. Third, maximal CO levels are seen close to the end of exhalation, as for CO_2. There is also a small proportion of CO derived from the airways, which is higher after allergen challenge measured either via bronchoscope (251), or at the mouth (104). The fact that breathing through the nose increases the CO levels obtained in the exhaled air (253) suggests that nose and paranasal sinuses may also contribute to the CO production of the human airways. Indeed, HO-like immunoreactivity is seen in the respiratory epithelium, in connection with seromucous glands and in the vascular smooth muscle of the nose (253).

Heme oxygenase. CO is a by-product of rate-limited oxidative cleavage of hemoglobin by HO, which exists in three iso-

forms, i.e., HO-1, HO-2, and HO-3. HO-2 is constitutively expressed in most tissues, whereas HO-3 is, so far, only described in rats (254). HO-1 has been identified as the major 32 kD heat shock (stress) protein (255). Like other stress proteins HO-1 can be induced by a variety of stimuli, such as proinflammatory cytokines, bacterial toxins, heme, ozone, hyperoxia, hypoxia, reactive oxygen species, and reactive nitrogen species. Both HO-1 and HO-2 are expressed in human airways and are found in most cell types, with particularly strong immunfluorescence in airway epithelial cells (256). Heme is converted by HO to biliverdin and thence to bilirubin, with the formation of CO and ferritin (Figure 4).

Interactions with NO. Like NO, CO is also capable of upregulating cyclic guanine monophosphate (cGMP) via activation of guanylyl cyclase causing vasodilatation, smooth-muscle relaxation, and platelet disaggregation. The vasodilatory effect of CO may be important in maintaining adequate tissue oxygenation and perfusion in the lung during normal physiology and in hypoxic conditions that result from pulmonary vascular diseases and acute lung injury. It has been suggested that the HO pathway exerts important counter-regulatory effects on the NOS pathway and, when blocked, the underlying NOS pathway is unmasked leading to increased and prolonged release of NO (257). In contrast, exogenously administered or endogenously released NO stimulates HO-1 gene expression and CO production in vascular smooth muscle cells resulting in a higher resistance to oxidant damage (258). This effect of NO is related to the release of free heme from heme proteins, which are able to transcriptionally up-regulate HO-1 and lead to their own degradation. CO also directly inhibits NOS2 activity by binding to the heme moiety of the enzyme (259). The effect of hemoglobin scavenging, as a function of the extent of bronchial arterial neovascularization (e.g. bronchiectasis, thromboembolic disease) may play an important role in the reaction between erythrocytic hemoglobin and NO. This interaction has been generally considered in the context of mechanisms that safely detoxify NO. More recently, hemoglobin-dependent mechanisms that preserve, not destroy, NO bioactivity *in vivo* have also been proposed (260). The emerging picture suggests that the interplay between NO and erythrocytic hemoglobin is important in regulating the functions of both these molecules *in vivo*. Hemoglobins modified for therapeutic use as either hemoglobin-based oxygen carriers or scavengers of nitric oxide are currently being evaluated in clinical trials. One such product, pyridoxalated hemoglobin polyoxyethylene conjugate (PHP), is a human-derived and chemically modified hemoglobin that has been successfully studied in Phase II clinical trials, and may be used for the treatment of shock associated with the systemic inflammatory response syndrome (261). The redox activity of modified hemoglobins can be attenuated, so that modified hemoglobins containing endogenous antioxidants such as PHP may have reduced pro-oxidant potential. These antioxidant properties, in addition to the NO-scavenging properties, may allow the use of PHP in other indications in which excess NO, superoxide, or hydrogen peroxide is involved, including severe asthma, CF, COPD, and bronchiectasis.

Effect of oxidative stress. There is a close link between the reactive oxygen and nitrogen species and CO. Thus, a dose-dependent increase in exhaled CO has been shown after a 1-h exposure to different concentrations of O_2 (262). HO-1 activation can be diminished by *N*-acetylcysteine, a precursor of glutathione with antioxidant properties (263). Both, superoxide anions and peroxynitrite can stimulate HO-1 activation (264), and subsequent release of CO is an important negative-feedback regulatory mechanism limiting the release of these cytotoxic substances (265). Animals exposed to a low concentration of CO exhibit a marked tolerance of the lungs to lethal concentrations of hyperoxia *in vivo* (266).

The precise mechanisms for this protection are not fully understood, but both the degradation of heme (with removal of iron and induction of ferritin) and the generation of bilirubin (an antioxidant) may be involved. There is evidence that the deleterious effects of ROS, such as superoxide and H_2O_2, are dependent on the presence of iron. The intracellular pool of free iron can react with both H_2O_2 and superoxide, giving rise to the OH^- radical via the Fenton reaction. The free iron that is not metabolized intracellularly sequestered in cells as ferritin. Thus, ferritin serves as a reservoir to restrict iron from participating in the Fenton reaction. It has been shown that free iron released from heme by HO may induce ferritin synthesis, and heme-induced HO-1 protein also activates ferritin via mRNA expression (267). Furthermore, the metabolite of heme degradation, bilirubin, is itself an effective antioxidant of peroxynitrite-mediated protein oxidation and may be even more effective than vitamin E in preventing lipid peroxidation (268). Moderate overexpression of HO-1 improves the resistance of cells to oxygen toxicity (269). However, there is cytotoxicity associated with HO-1 overexpression.

HO-2 may also protect against oxidative stress. HO-2 knockout mice are sensitized to hyperoxia-induced oxidative injury,

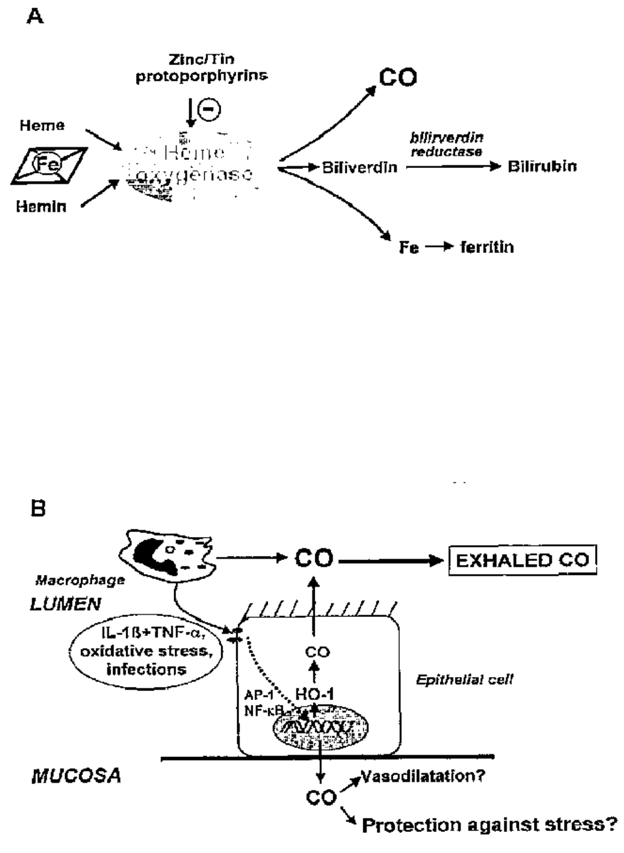

Figure 4. Synthesis of carbon monoxide (CO) (*panel A*). Sources of CO in exhaled air (*panel B*).

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have a higher mortality, and increased lung iron content without increased ferritin, suggesting accumulation of available redox-active iron (270).

Measurement

Exhaled CO as a marker to assess different diseases (cardiovascular, diabetes, and nephritis) was first described in Russia 1972 (271). Over the last 20 yr exhaled CO has been measured to identify current and passive smokers, to monitor bilirubin production, including hyperbilirubinemia in newborns, and in the assessment of the lung diffusion capacity.

CO can be quantified by a number of different techniques. Most of the measurements in humans have been made using electrochemical CO sensors. The sensor is selective, gives reproducible results (272), and is inexpensive. However, these instruments are susceptible to interference from a large number of substances, for example, hydrogen, which is present in exhaled breath and may be increased after glucose ingestion. H_2-insensitive CO sensors, which are now available, are therefore recommended.

Exhaled CO can also be measured (at ppb level) by adjustable laser spectrophotometer (262, 273), or by a near-infrared CO analyzer (274). Near-infrared instruments, are used for continuous monitoring of atmospheric CO, and are fairly sensitive and stable. However, they are larger than electrochemical CO sensors, sensitive to water and CO_2 concentrations, and require large sample volumes (275). This may explain the low CO levels detected by these instruments even after a prolonged breathhold time of 20s (274). Gas chromatography is a reference method for CO measurements, but its use is limited to specialized laboratories.

End-tidal exhaled CO measurements can be made during a single exhalation and is a routine in cooperative adults. It can also be easily performed in children older than 5 yr of age (276). A method for measuring CO in nasally sampled exhaled air in noncooperative neonates has been developed that involves the relatively noninvasive placement of a small catheter into the posterior of the nasopharynx and the collection of breath samples either manually or automatically (249).

Factors Affecting Exhaled CO Measurements

CO exists in the atmosphere as a by-product of incomplete combustion and oxidation of hydrocarbons, and is oxidized to CO_2 by hydroxyl radicals, or eliminated either by soil microorganisms or by stratospheric diffusion. Regional and local levels of CO in ambient air can vary significantly depending on time of the day and season, on wind velocity, industrialization, traffic, and altitude. Although some exposure to CO may occur in normal day-to-day life because of environmental pollution, active or passive smoking are the most likely reason for high levels of exhaled CO. After inhalation, CO displaces oxygen in the erythrocyte to form carboxyhemoglobin (COHb), which has a half-life of about 5 to 6 h in this form. A cutoff level of 6 ppm (277) effectively separates nonsmokers from smokers, and the previously used cutoff 8 ppm (278) or 10 ppm (279) may be too high. Other individual factors, which can markedly affect the amount of CO that a person may inhale, are type and location of home and occupation, cooking/heating appliances, and mode of transportation.

Many pathologic conditions and factors can increase the rate of hemoprotein breakdown and potentially increase the levels of exhaled CO, including anemias, hematomas, and preeclampsia. Nonpathologic factors may also increase endogenous CO production, including fasting, dehydration, some drugs (phenobarbitone), and xenobiotic compounds (paint remover) (280) (Table 2).

Asthma

Elevated levels of exhaled CO have been reported in stable asthma (281, 282) with normal levels in patients treated with inhaled corticosteroids (282). The difference in exhaled CO between normal and asthmatic subjects, however, is much less than in exhaled NO (283), and the effect of inhaled steroids on exhaled CO in patients with mild asthma, as it has been reported recently, is negligible (256). Both HO-1 and HO-2 are extensively distributed in airways of normal and asthmatic subjects (256). The increased levels in stable asthma are likely to be due to preferential increase of HO-1 expression, which is seen in alveolar macrophages in induced sputum of patients with asthma (263). There is also an increase in the concentration of bilirubin in induced sputum, indicating increased HO-1 activity (263). Further evidence that exhaled CO increases may reflect HO activity is the demonstration that inhaled hemin, which is a substrate for HO, results in a significant increase in exhaled CO concentration in normal and asthmatic subjects (263). Increased levels of exhaled CO are seen in acute exacerbations of asthma, and are reduced after treatment with oral corticosteroids (284). Significantly elevated CO levels are found in patients with severe asthma (285), including patients treated with 30 mg of prednisolone for 2 wk (286). In view of the simplicity of CO measurements and the portability of CO analyzers, exhaled CO may be useful in noninvasive monitoring of pediatric asthma. For example, children with persistent asthma despite treatment with steroids, which reduce their NO levels, have significantly higher exhaled CO than do those with infrequent episodic asthma (276).

COPD

A major limitation of exhaled CO in COPD is the marked effects of cigarette smoking, which masks any increase that may occur because of the disease process. There is no difference in exhaled CO in patients with chronic bronchitis (without airflow obstruction) when compared with normal subjects (287). However, exhaled CO levels are elevated in ex-smoking patients with COPD (288), suggesting ongoing oxidative stress or inflammation. HO is induced in fibroblasts exposed to cigarette smoke (289). There is an increase in exhaled CO during acute exacerbations of COPD, with a decline after recovery (290).

Bronchiectasis

Exhaled CO levels are elevated in patients with bronchiectasis, irrespective of whether they are treated with inhaled corticosteroids (291).

Cystic Fibrosis

In contrast to NO, exhaled CO levels were markedly elevated in patients with stable CF (292–294) and increased further during exacerbations and reduced with antibacterial treatment (Figure 5) (176). This suggests that exhaled CO is not only a marker of oxidative stress/inflammation in CF, but is also a marker of disease severity. This is further confirmed by the finding of lower CO levels in patients receiving oral corticosteroid treatment (292–294). In fact, by reducing airway inflammation and the release of oxidants by inflammatory cells steroids may attenuate HO-1 expression and the synthesis of CO. We have shown that patients homozygous for the CF transmembrane regulator ΔF508 mutation have higher exhaled CO levels than do heterozygous patients (292). Considering the growing interest in gene therapy in cystic fibrosis, further studies are needed to investigate the role of CO levels in the assessment of effective therapeutic gene delivery or to

confirm the diagnosis in patients with borderline sweat tests where more extensive genetic analysis is not available.

Interstitial Lung Disease

Elevation of exhaled CO is related to lung function deterioration (295) and impaired gas transfer in patients with cryptogenic fibrosing alveolitis and scleroderma (296). Elevated levels of exhaled CO in patients with fibrosing alveolitis are also associated with disease activity as assessed by BAL cell counts (212). This suggests that exhaled CO may be used to monitor disease progression and response to therapy in interstitial lung diseases.

Allergic Rhinitis

Stable levels of CO are recorded during continuous sampling from one nostril during normal breathing through the mouth in normal subjects (253). Sampling through a drainage tube inserted into the maxillary sinus reveals CO levels comparable to the levels obtained by sampling through the nose. In patients with allergic rhinitis exhaled CO is increased during the pollen season and returns to normal values after the season (297). The levels of exhaled CO are significantly higher in patients with symptoms than in those without. However, there is no correlation between nasal and exhaled samples, suggesting that the increase is derived from the lower respiratory tract. We did not measure any direct nasal CO production in either normal or asthmatic subjects (283).

Infections

HO-1 is induced by many infectious agents, and HO-1 may provide protection to cells against attack by infectious agents. Upper respiratory tract viral infections may induce the expression of HO-1, resulting in increased exhaled CO in adults (298) and in children (276). Elevated exhaled CO levels might provide an early warning signal for an acute infective episode, which may lead to exacerbation of asthma and COPD. Elevated levels of CO have been measured in patients in general practice with lower respiratory tract infection, which were significantly reduced after 5 d of treatment with antibiotics (290).

TABLE 2. FACTORS INFLUENCING EXHALED CO

Exhaled CO	Reference
Miscellaneous	
↑ Smoking	(277, 465)
↑ Airway pollution	(466, 467)
↑Airway obstruction	(468)
↑ Hyperbilirubinemia	(469)
Sex (cyclic variations in women)	(470)
Race (↑ COHb in Japanese newborn)	(471)
Disease	
↑ Allergen challenge (early and late response)	(104)
↑ Asthma (mild-moderate)	(281, 282, 284)
↔ Asthma (mild)	(285)
↑Asthma (severe)	(285)
↑Atopy	(101)
↑ Asthma in children (persistant asthma)	(276)
↑ Allergic rhinitis	(297)
↑ COPD (ex-smokers)	(288)
↑ Upper respiratory tract infections	(276, 298)
↑ Bronchiectasis and lower respiratory tract infections	(290, 291)
↑ Interstitial lung disease	(295)
↑ CF	(176, 292–294)
↑ Citically ill patients	(299)

Definition of abbreviations: ↓ = decrease; ↑ = increase; ↔ = no change.

Other Conditions

Critically ill patients have a significantly higher CO concentration in exhaled air as well as total CO production than do healthy control subjects (299), but inspired oxygen concentration has to be measured, as it can influence CO excretion in mechanically ventilated patients (300). Interestingly, the levels of exhaled CO in these patients are similar to the levels seen in severe asthma and may be a reflection of systemic rather than the local oxidative stress.

Exhaled CO levels are also increased in diabetes, and the level is significantly related to the level of hyperglycemia (301). The mechanism is unclear, but hyperglycemia and oxidative stress in uncontrolled diabetes may activate HO-1.

EXHALED HYDROCARBONS

Almost 30 yr ago Pauling and co-workers (302) reported that normal human breath contains a mixture of several hundred volatile organic compounds. Exhaled hydrocarbons have been measured in a variety of conditions, ranging from the monitoring of lipid peroxidation in cosmonauts during long-term space flights (303) to patients undergoing cardiopulmonary bypass operations (304). Hydrocarbons are non-specific markers of lipid peroxidation, which is one of the consequences of the constant and inevitable formation of oxygen radicals in the body. During the process of peroxidation of polyunsaturated fatty acids hydrocarbons are distributed in the body, partly metabolized, and excreted in the breath, making it possible to estimate the magnitude of *in vivo* lipid peroxidation. Numerous methods have been developed to measure lipid peroxidation products and lipid peroxidation damage in tissues, cells, and body fluids. For volatile organic compounds, sampling and analysis of breath is preferable to direct measurement from blood samples because it is noninvasive, and the measurements are much simpler in the gas phase than in a complex biologic fluid. Recently, in patients with abnormal chest radiographs, a combination of 22 volatile organic compounds discriminated patients with and without lung cancer (305), suggesting that exhaled breath profile of hydrocarbons may be more informative than single hydrocarbons.

Origin

In contrast to the predominantly airway source of exhaled NO, hydrocarbons are representative of blood-borne concentrations through gas exchange in the blood/breath interface in the lungs. The main source of exhaled hydrocarbons in the body is the liver (306), with contribution from red blood cells and other organs (307). The low molecular mass hydrocarbons ethane and pentane are among the numerous end-products of lipid peroxidation of peroxidized polyunsaturated fatty acids, and have been extensively studied in exhaled breath. How-

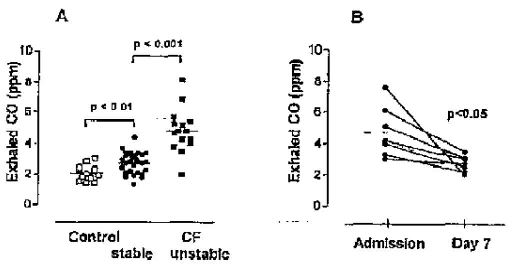

Figure 5. Exhaled CO in cystic fibrosis (CF): disease severity (*panel A*) and effect of anti-microbial treatment (*panel B*) (from Reference 176).

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ever, the primary localization of their generation it is not yet clear. Hydrocarbons such as propane and butane (products of peroxidation of linoleic and arachidonic acid) are mainly derived from protein oxidation and fecal flora and their role as the markers of lipid peroxidation is doubtful. However, ethane and pentane excretion are increased during the first few days of life in premature newborns when the gut is not colonized and, therefore, supporting that the bacterial flora is not the major contributor of these exhaled hydrocarbons (308). The available evidence suggests that peroxidation of polyunsaturated fatty acids is the major, if not the only, endogenous source of the pentane and ethane in breath (307).

Measurement

The first reports of exhaled breath analysis using gas chromatography go back more than 30 yr (309), and since then lipid peroxidation has gained increasing interest as one of the more prominent features of free-radical-induced damage in clinical medicine. Lipid peroxidation is assessed by measuring its secondary reaction products such as chemiluminescent and fluorescent molecular products, lipid hydroperoxides, conjugated dienes, aldehydes, malonaldehyde or thiobarbituric acid-reactive substances, and aliphatic hydrocarbons (307). Exhaled hydrocarbons are measured by gas chromatography. There are several technical difficulties that should be overcome to obtain reliable measurements. Sample preparation and storage were major problems in earlier methods for breath analysis. Recently, avoidance of air contamination, adequate preinjection concentrations of the samples, and sensitive gas chromatographic techniques have enabled more accurate and reproducible measurements of hydrocarbons in human breath (310–312). New techniques have been also developed for analyzing small volumes of gas (ethane, pentane) from single-breath samples, in which no preconcentration is required (313) and exhaled air, collected during a single flow-controlled exhalation into a Teflon reservoir, is injected directly into gas chromatograph (294, 314, 315). Particular attention should be paid to the storage (no longer than 48 h in Tedlar bags and 24 h in capped desorption tubes). Measurement of exhaled pentane is more problematic than ethane, as it exists in the ambient air and is coeluted with isoprene, a prominent hydrocarbon that is affected by diet (316). The presence of specific compounds, for example, ethane and pentane in the breath, may also be an indicator of recent exposure to the gases. The contamination of ambient ethane can be eliminated by discarding the dead space during the first part of exhalation, and potential loss of organic vapors to condensed water is excluded by using silica gel granules in reservoirs (294, 314, 315, 317). A simple field method for sampling benzene in end-exhaled air of healthy subjects has been developed, where the sample is collected directly on an adsorbent tube while the subject exhales through the sampling device, which consists of a modified peak expiratory meter (318). Exhaled breath condensate is another source of the lipid fatty-acids, which can be analyzed by gas chromatography, as described in children with pneumonia (319, 320).

Factors Affecting Levels in Exhaled Air

Hydrocarbons are present in ambient air, inhaled and retained in steady state in the equilibrium between various body compartments (body fat) and ambient air. Although it takes a few minutes to wash out the lungs, it requires no less than 90 min to wash out the body stores of hydrocarbons, making this approach impractical (321). To make exhaled breath hydrocarbon tests usable in clinical medicine two approaches to deal with ambient contamination have a considerable advantage (307). First employment of a washout period (4 to 10 min), and second to record the local ambient levels of hydrocarbons and subtract them from the levels in exhaled breath (294).

Newborns excrete ethane 11-fold and pentane 12-fold more than healthy adult men (322). The elimination of hydrocarbons is mainly (85%) the results of metabolism by hepatic cytochrome P450 enzymes (323). Therefore, large doses of ethanol, or other liver toxic agents (acetone) may increase pentane in exhaled air because of P-450 inhibition. Exhaled pentane in infants receiving total parenteral nutrition, including intravenous lipid emulsion, is 70 times higher than in adults (322). However, no statistically significant changes in exhaled hydrocarbons are found relative to the fasting level, suggesting that diet does not alter ethane or pentane excretion in healthy subjects (324).

Although different minute volumes has no effect on ethane excretion in children (325), the diffusion rate of lipophilic substances such as ethane and pentane may be reduced and will require a longer exhalation, or collection of the last part of exhalation (294). Smoking increases exhaled ethane and pentane. This effect is possibly related to oxidative damage caused by smoking and to high concentrations of hydrocarbons in cigarette smoke (326–328). Both mental (329) and physical stress (330) also increases lipid peroxidation levels of ethane and pentane in exhaled air of normal subjects.

Oxidative stress causing cell damage and lipid peroxidation plays an important role in several inflammatory lung diseases such as COPD, asthma, CF, and interstitial lung disease. Exhaled hydrocarbons may help to estimate the magnitude of *in vivo* lipid peroxidation by measuring, for example, pentane and ethane exhaled in breath, and to monitor the effect of novel drugs with antioxidant properties in clinical practice.

Asthma

Exhaled pentane is elevated during acute asthma exacerbations and reduced to normal levels during recovery (331). Exhaled ethane levels are higher in patients with mild steroid-naïve asthma compared with steroid-treated patients and normal subjects (332) (Figure 6). The measurements of two different exhaled markers, NO and pentane for example, might be helpful to distinguish severe nocturnal asthma from obstructive sleep apnea, which is associated with low levels of circulating nitrite/nitrate (333). Elevated levels of exhaled and nasal NO, but not pentane, have been found in patients with sleep apnea (334), suggesting the presence of predominantly upper airway inflammation in these patients.

COPD

Pentane (335) and isoprene (336) are increased in normal smokers (328), and ethane in patients with COPD who smoke (327) (Figure 6). Although vitamin E given for 3 wk failed to reduce exhaled ethane in cigarette smokers, those whose ethane values fell the most tended to have better-preserved lung function (337). Increased levels of volatile organic compounds in exhaled breath could be used as biochemical markers of exposure to cigarette smoke and oxidative damage caused by smoking. For example, levels of 2,5-dimethyl furan (338), or known carcinogen benzene (339), in smokers are sufficiently discriminative to differentiate smokers from nonsmokers. However, if transient elevation of ethane in exhaled air (returned to baseline within 3 h) in healthy smokers is due to ethane in cigarette smoke, chronically elevated ethane levels in current and, especially in ex-smokers, is more likely related to oxidative damage (326). In fact, there is a correlation between the ethane levels and the degree of airway obstruction in COPD (327), and current (packs per day) and lifelong (pack-years) tobacco consumption (328). Breath analysis, therefore, may also be employed to evaluate the elimination process of a variety of vola-

tile organic compounds after microenvironmental exposures, and an improved portable breath measurement method has been successfully tested (340).

Cystic Fibrosis

Patients with CF have elevated levels of exhaled ethane, which is significantly correlated with exhaled CO and airway obstruction (294) (Figure 6), supporting the view that oxidative stress and lipid peroxidation are increased in the airways of patients with CF.

Other Lung Diseases

Exhaled breath profile of different hydrocarbons may be of diagnostic value in a variety of clinical conditions, as it has been shown in patients with lung cancer (305). Simultaneous pentane and isoprene measurements have been measured in critically ill mechanically ventilated patients (341). In patients who developed pulmonary infection, pentane elimination was increased, but isoprene elimination was reduced, resulting in a significant increase in their ratio when compared with patients without pulmonary infection. A significant increase of exhaled ethane, which is related to a lower cardiac index and a higher systemic vascular resistance, has been demonstrated in patients undergoing cardiopulmonary bypass operations (304), suggesting oxidative damage caused by reperfusion in these patients. A potentially important application for exhaled hydrocarbons analysis would be to differentiate patients with viral and bacterial infection to justify the use of antibiotics. Elevated levels of pentane are found in critically ill patients who develop chest infection compared with patients without pulmonary infection, and they might be an indication for antibiotic treatment (341). It might even be possible, in the future, to identify a specific pathogen, hence to apply the most appropriate antibiotic therapy by studying the patients' exhaled hydrocarbon profiles.

EXHALED BREATH CONDENSATE

The detection of nonvolatile mediators and inflammatory markers from the respiratory tract involves invasive techniques such as bronchoalveolar lavage or induced sputum. They cannot be repeated within a short period of time because of their invasiveness, and because the procedures themselves may induce an inflammatory response (2, 342). Exhaled breath condensate is collected by cooling or freezing exhaled air and is totally noninvasive. The collection procedure has no influence on airway function or inflammation, and there is accumulating evidence that abnormalities in condensate composition may reflect biochemical changes of airway lining fluid. Several nonvolatile chemicals, including proteins, have now been detected in breath condensates. The first studies identifying surface-active properties, including pulmonary surfactant, of exhaled condensate were published in Russia in the 1980s (343, 344) and since then several inflammatory mediators, oxidants, and ions have been identified in exhaled breath condensates.

Origin

Potentially, condensate measurements reflect different markers and molecules derived from the mouth (oral cavity and oropharynx), tracheobronchial system, and alveoli, and their proportional contribution has not yet been sufficiently studied. It is assumed that airway surface liquid becomes aerosolized during turbulent airflow, so that the content of the condensate reflects the composition of airway surface liquid, although large molecules may not aerosolize as well as small soluble molecules. A strong correlation between the levels of CO_2 and O_2 in exhaled fluid and exhaled breath (345) suggests that aerosol particles exhaled in human breath reflect the composition of the bronchoalveolar extracellular lining fluid.

Factors Affecting Measurements

Several methods of condensate collection have been described. The most common approach is to ask the subject to breathe tidally via a mouthpiece through a non rebreathing valve in which inspiratory and expiratory air is separated (Figure 7). During expiration the exhaled air flows through a condenser, which is cooled to 0° C by melting ice (346), or to −20° C by a refrigerated circuit (347), and breath condensate is then collected into a cooled collection vessel. A low temperature may be important for preserving labile markers as lipid mediators during the collection period, which usually takes between 10 to 15 min to obtain 1 to 3 ml of condensate. Exhaled condensate may be stored at −70° C and is subsequently analyzed by gas chromatography and/or extraction spectrophotometry, or by immunoassays (ELISA).

Salivary contamination may influence the levels of several markers detectable in exhaled breath condensate. Thus, high concentrations of eicosanoids (thromboxane B_2, LTB_4, $PGF_{2\alpha}$), but low levels of PGE_2 and prostacyclin have been found in saliva of children with acute asthma (348). The presence of high concentrations of nitrite/nitrate from the diet may affect NO-related markers in condensate (349). It is therefore important to minimize and monitor salivary contamination. Subjects should rinse their mouth before collection and to keep the mouth dry by periodically swallowing their saliva. Salivary contamination, measured by amylase concentration of condensate, should be routinely monitored. In most of the studies reported, amylase has been measured in condensate and no salivary contamination has been detected (347, 350, 351). Subjects should wear a noseclip in order to collect only mouth-conditioned exhaled air into the collection system. Flushing the nose with helium may help to reduce contamination of ex-

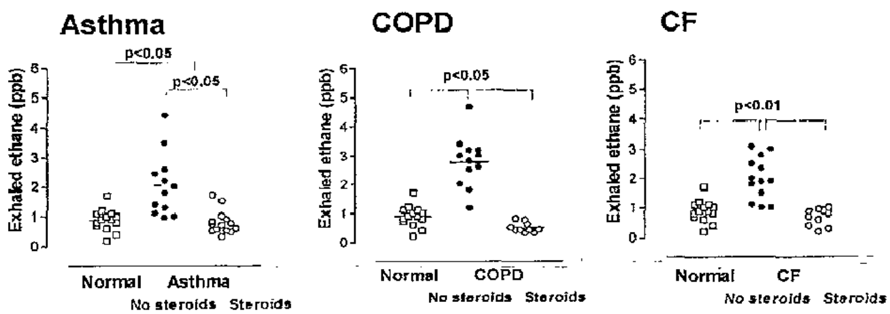

Figure 6. Exhaled ethane in asthma (from Reference 332), COPD (from Reference 327), and CF (from Reference 294).

haled breath with nasal air that contains high levels of NO, which potentially may influence the results of NO-related markers (nitrite/nitrate, *S*-nitrosothiols) (352).

Another approach to exclude nasal contamination is to collect condensate during a series of exhalations against a resistance (352). However, it has not yet been shown that nasal NO affects measurements in exhaled condensate. The quantity of exhaled condensate is dependent on the ventilation volume per unit time (minute volume), but this does not affect the concentration of mediators (346, 353). It is also dependent on exhaled air temperature and humidity (Paredi P, *et al.*: unpublished observation).

Hydrogen Peroxide

Activation of inflammatory cells, including neutrophils, macrophages, and eosinophils, result in an increased production of O_2^-, which by undergoing spontaneous or enzyme-catalyzed dismutation lead to formation of H_2O_2. As H_2O_2 is less reactive than other reactive oxygen species, it has the propensity to cross biologic membranes and enter other compartments (354). Because it is soluble, increased H_2O_2 in the airway equilibrates with air (355). Compared with the cellular antioxidant scavenging systems, the extracellular space and airways have significantly less ability to scavenge reactive oxygen species (356, 357). Catalase is the major enzyme involved in removing H_2O_2 and is preset in low concentrations in the respiratory tract. Thus, exhaled H_2O_2 has potential as a marker of oxidative stress in the lungs.

Asthma. H_2O_2 has been detected in exhaled condensate in healthy adults and children with increased concentrations in asthma (350, 355, 358, 359). There is no correlation between the levels of exhaled H_2O_2 and age, sex, or lung function in healthy children (359). However, exhaled H_2O_2 concentration is related to the number of sputum eosinophils and airway hyperresponsiveness in asthma of different severity, and it is elevated in patients with severe unstable asthma, although exhaled NO is significantly reduced by treatment with corticosteroids (350). This may be related to the fact that neutrophils, prevalent in severe asthma (121), generate higher amounts of superoxide radicals and therefore H_2O_2 (360). Asthmatic patients also exhale significantly higher levels of thiobarbituric acid-reactive products (TBARs), which indirectly reflect increased oxidative stress (358).

COPD. Cigarette smoking causes an influx of neutrophils and other inflammatory cells into the lower airways, and fivefold higher levels of H_2O_2 have been found in exhaled breath condensate of smokers than in nonsmokers (361). Levels of exhaled H_2O_2 are increased compared with those in normal subjects in patients with stable COPD and are further increased during exacerbations (362, 363). Cigarette smoking is by far the commonest cause of COPD, but only 10 to 20% of smokers develop symptomatic COPD. No significant differences have been found between H_2O_2 levels in current smokers with COPD and subjects with COPD who have never smoked, and there is no correlation between expired H_2O_2 concentration and daily cigarette consumption (363). Thus, oxidative stress is a characteristic feature of COPD and presumably related to airway inflammation, and it cannot be explained entirely by the oxidants present in tobacco smoke.

Other lung diseases. Increased H_2O_2 levels in exhaled breath condensate have been found in ARDS (364, 365), bronchiectasis (351), and after lobectony/pneumonectomy in patients with lung carcinoma (366), indicating increased oxidative stress in these conditions, and are significantly reduced during antibiotic treatment in patients with infective exacerbations of CF (367).

Eicosanoids

Eicosanoids are potent mediators of inflammation responsible for vasodilatation/vasoconstriction, plasma exudation, mucus secretion, bronchoconstriction/bronchodilatation, cough, and inflammatory cell recruitment. They are derived from arachidonic acid and include prostaglandins, thromboxane, isoprostanes and leukotrienes. Noninvasive exhaled condensate analysis provides an opportunity to assess the eicosanoid profile in lung diseases directly, and it may be a better predictor of clinical efficacy of leukotriene antagonists or thromboxane inhibitors in lung disease than urine, serum, or invasive BAL.

Prostanoids. There is an increased expression of inducible cyclooxygenase (COX-2), which forms prostaglandins and thromboxane in asthma and COPD (368) and CF (369). Most prostaglandins and thromboxane have proinflammatory properties, but others, for example PGE_2 and PGI_2, are anti-inflammatory (370). For example, PGE_2 inhibits induction of NOS2 in cell lines (371), and when inhaled reduces exhaled NO in asthma (155). Exhaled prostanoids are detectable in exhaled breath condensate. PGE_2 and $PGF_{2\alpha}$ are markedly increased in patients with COPD, whereas these prostaglandins are not significantly elevated in asthma (372). In contrast, TxB_2 is increased in asthma but not detectable in normal subjects or in patients with COPD (Montuschi P. *et al.*, unpublished observation). Exhaled thromboxanes may predict more

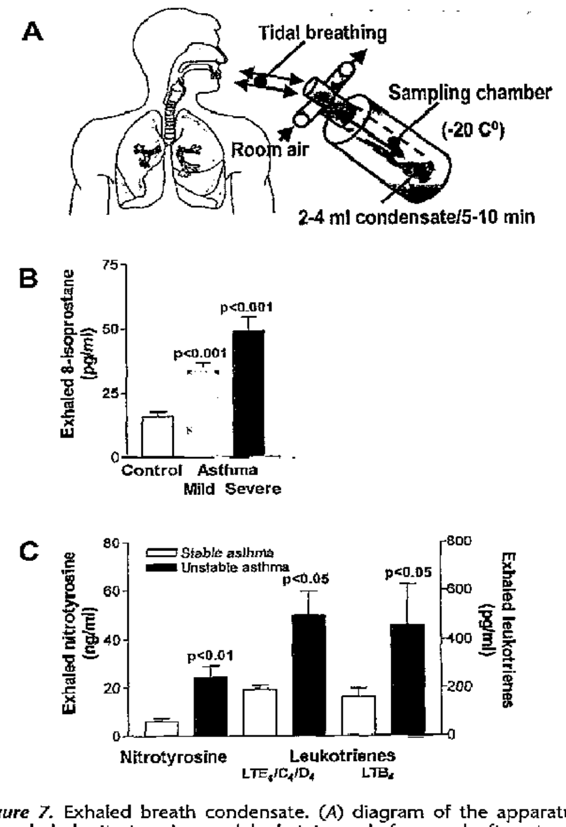

Figure 7. Exhaled breath condensate. (*A*) diagram of the apparatus. (*B*) exhaled nitrotyrosine and leukoteienes before and after steroid withdrawal in patients with moderate asthma (from Reference 381). (*C*) exhaled 8-isoprostane in asthma (from Reference 346).

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accurately than urinary levels those patients who may benefit from a thromboxane receptor antagonist in asthma (373).

Leukotrienes. Leukotrienes (LTs), a family of lipid mediators derived from arachidonic acid via the 5-lipoxygenase pathways, are potent constrictor and proinflammatory mediators that contribute to the pathophysiology of asthma. The cysteinyl-leukotrienes (cys-LTs) LTC_4, LTD_4 and LTE_4 are generated predominantly by mast cells and eosinophils and are able to contract airway smooth muscle, cause plasma exudation, and stimulate mucus secretion, as well as recruiting eosinophils (374). By contrast, LTB_4 has potent chemotactic activity towards neutrophils (375). Detectable levels of LTB_4, C_4, D_4, E_4, and F_4 have been reported in exhaled condensate of asthmatic and normal subjects (376, 377). Elevated exhaled condensate levels of LTB_4 have been found in healthy calves during an experimental chest infection (353). There have been attempts to measure leukotrienes in urine, and increased levels of LTE_4 have been reported in some asthmatic patients, but they are not consistently increased after allergen challenge (378). Allergen provocation increases LTC_4 and LTE_4 concentrations in BAL and in urine during early and late asthmatic responses (379). However, measurement of airway mediators in urine is problematic because of dilution of the lung-derived signal and delay in excretion. Increased levels of LTE_4 have also been found in induced sputum during the late response to allergen in patients with mild asthma (380). In patients with mild asthma levels of LTE_4, LTC_4, LTD_4 in exhaled condensate are increased during the late asthmatic response to allergen challenge (381). The levels of leukotrienes LTE_4, C_4, D_4 in breath condensate are elevated significantly in patients with moderate or severe asthma (377), and steroid withdrawal in moderate asthma leads to worsening of asthma and further increase in exhaled NO and the concentration of LTB_4, LTE_4, LTC_4, LTD_4 in exhaled condensate (381) (Figure 7). LTB_4 concentrations are increased in exhaled breath condensate of patients with COPD (Montuschi P. *et al.*: unpublished observations) and in moderate and severe asthma (377). This suggests that LTB_4 may be involved in exacerbations of asthma and may contribute towards neutrophils recruitment.

Isoprostanes. Isoprostanes are a novel class of prostanoids formed by free radical-catalyzed lipid peroxidation of arachidonic acid (382). They are initially esterified in membrane phospholipids, from which they are cleaved by a phospholipase A_2, circulate in plasma, and are excreted in urine, and can be detected in exhaled breath condensate and BAL. Their formation is largely independent of COX-1 and COX-2. They can be detected by ELISA (346, 383) and by GC/MS analysis (382). F_2-isoprostanes are the major candidates for clinical measurement of oxidative stress *in vivo*. They are stable compounds, detectable in all normal biologic fluids and tissues (384), and their formation is increased by systemic oxidative stress, for example in patients with diabetes (385) or ARDS (386). F_2-isoprostanes are reduced by antioxidants, for example by alpha-lipoic acid in normal subjects (387). They are not simply markers of lipid peroxidation but also possess biologic activity, and they could be mediators of the cellular effects of oxidant stress and a reflection of complex interactions between the RNS and ROS. Indeed, peroxynitrite is capable of activating biosynthesis of endoperoxide synthase and thromboxanes in inflammatory cells (388), and oxidizing arachidonic acid to form F_2-isoprostanes. The most prevalent isoprostane in humans in 8-epi-$PGF_{2\alpha}$, also known as 8-isoprostane.

Asthma. F_2-isoprostanes are increased in plasma (389) and BAL fluid of asthmatic patients and further increased after allergen challenge (390). 8-isoprostane levels are approximately doubled in patients with mild asthma compared with those in normal subjects, and increased by about 3-fold in those with severe asthma, irrespective of their treatment with corticosteroids (346) (Figure 7). The relationship to asthma severity is a useful aspect of this marker, in contrast to exhaled NO. The relative lack of effect of corticosteroids on exhaled 8-isoprostane has been confirmed in a placebo-controlled study with the two different doses of inhaled steroids (120). This provides evidence that inhaled corticosteroids may not be very effective in reducing oxidative stress. Exhaled isoprostanes may a better means of reflecting disease activity than exhaled NO.

COPD. Urinary levels of isoprostanes, in particular 8-isoprostane, are increased in COPD, but they decline in patients with acute exacerbation as their clinical condition improves (391). Aspirin treatment fails to decrease urinary levels of isoprostanes, whereas TxB_2 were significantly reduced, confirming that cyclooxygenases are not involved in their formation. The concentration of 8-isoprostane in exhaled condensate is also increased in normal cigarette smokers, but to a much greater extent in patients with COPD (392). Interestingly, exhaled 8-isoprostane is increased to a similar extent in patients with COPD who are ex-smokers as in smoking patients with COPD, indicating that the exhaled isoprostanes in COPD are largely derived from oxidative stress from airway inflammation, rather than from cigarette smoking.

Cystic fibrosis. CF is characterized by marked oxidative stress in the airways (393), and elevated levels of 8-isoprostane have been detected in plasma (394). Concentrations of 8-isoprostane in the breath condensate of patients with stable CF are increased about threefold compared with those in normal subjects (293).

Interstitial lung disease. Interstitial lung diseases such as cryptogenic fibrosing alveolitis (CFA) and fibrosing alveolitis associated with systemic sclerosis (FASSc), are characterized by enhanced oxidative stress in both serum (395) and BAL fluid (396). The imbalance between the oxidants and antioxidants is also a prominent feature of sarcoidosis (397). 8-isoprostane is detectable in BAL fluid of normal subjects and is increased in patients with sarcoidosis, CFA, and FASSc, suggesting a higher level of oxidant stress and greater lung injury in these patients than in those with sarcoidosis (383).

Products of Lipid Peroxidation

There are several methods to measure lipid peroxidation products and lipid peroxidation damage in tissues, cells, and body fluids. The most simple, but nonspecific, method is measurement of thiobarbituric acid-reactive substances (TBARS). The specificity of colorimetric or fluorimetric assays can be significantly improved if combined with high pressure liquid chromatography. If levels of TBARS are increased, as for example in exhaled condensate in asthma and COPD (358), other more sophisticated assays may be performed for verification. Assays are available for phospholipid- and cholesterylester, hydroperoxides, aldehydic lipid peroxidation products, including 4-hydroxynonenal, fluorescent protein adducts (e.g., lipofuscin), conjugated dienes, and antioxidants (398).

Although there is a still a question whether lipid peroxidation contributes to organ dysfunction or simply reflects oxidative injury, tissue-specific lipid peroxidation has been confirmed. Thus, lung lipid conjugated dienes are increased after intravenous infusion of both endotoxin and H_2O_2 in rats (399). However, venous plasma-conjugated dienes are elevated only after H_2O_2. Significantly higher concentrations of primary (diene conjugates) and secondary (ketodienes) products of lipid peroxidation have been found in exhaled condensate and in bronchial biopsy samples from patients with COPD and chronic bronchitis compared with those in normal subjects (400, 401).

Increased levels of free fatty acids, including linoleic and arachidonic acids, have been measured in exhaled condensate and sweat in children (320) and in adults (402) with acute pneumonia and lung edema (403). In contrast, the level of lipid peroxidation in patients with cancer was significantly reduced compare with that in healthy control subjects (404). Exhaled condensates may be used in prenatal diagnosis of fetal hypoxia, as significantly higher levels of diene conjugates and malonic dialdehydes have been found in pregnant women who gave birth to babies with severe fetal and neonatal hypoxia (405). Recent studies have suggested that the increased permeability in patients with interstitial lung disease results in an increase of alveolar-to-vascular leakage of surfactant proteins A and D (406). The clearance system of these proteins from the bloodstream is unknown at present, but if they are detectable in exhaled breath condensate, they may be the best practical examination for this disease.

Vasoactive Amines

Elevated levels of acetylcholine, serotonin, and histamine, which were related to the severity of airway inflammation, airway obstruction, and airway hyperresponsiveness, have been reported in exhaled breath condensate in asthma (407) and in acute bronchitis (408). High levels of acetylcholine, catecholamines, histamine, and serotonin and low levels of cortisol and thyroxine are reported in exhaled condensate in coal miners with early stages of silicosis (409).

NO-related Products

NO reacts with superoxide to yield peroxynitrite, and it can be trapped by thiol-containing biomolecules such as cysteine and glutathione, to form *S*-nitrosothiols or can be oxidized to nitrate and nitrite (410). Nitrogen intermediates, for example peroxynitrite, can induce a number of covalent modifications in various biomolecules such as nitrosoadducts and nitroadducts. One such modification yields 3-nitrotyrosine, and detection of this adduct in proteins is now commonly used as a diagnostic tool to identify involvement of NO-derived oxidants in many disease states (411). The balance between nitrite/nitrate, *S*-nitrosothiols, and nitrotyrosine in lung epithelial lining fluids, as reflected by exhaled breath condensate, gives insight into NO synthesis and short- and long-term changes in NO production. There are several methods, apart from the immunoassays, available for nitrite/nitrate and *S*-nitrosothiol quantification. They include an adsorptive stripping voltammetry (412) and electrochemical (413), fluorimetric, and colorimetric measurements (414, 415). There is also a method that allows the separation of the thiols from their *S*-nitrosylated derivatives using capillary zone electrophoresis (416).

Asthma. High levels of nitrite have been found in exhaled breath condensate (417) and sputum (418) of asthmatic patients, especially during acute exacerbations (417). The ratio of airway wall thickness to lumen diameter measured by high resolution computed tomography was significantly correlated with the sputum concentration of nitrite/nitrate (418). In fact, we have shown that nitrotyrosine, a stable product of peroxynitrite decomposition in exhaled breath condensate, is increased in mild steroid-naïve asthma and is reduced in patients with severe asthma receiving steroid therapy (377). However, increased levels of nitrotyrosine in exhaled breath condensate are associated with worsening of asthma symptoms and deterioration of lung function during inhaled steroid withdrawal in moderate asthma (381), suggesting that nitrotyrosine may be not only a predictor of asthma deterioration, but may play a key role in the pathogenesis of airway remodeling.

A deficiency in *S*-nitrosothiols has been demonstrated in tracheal lining fluid in asthmatic children with respiratory failure (419), suggesting that the levels of *S*-nitrosothiols, which are endogenous bronchodilators, may normally counteract increased airway tone in asthma. The levels of *S*-nitrosothiols in exhaled breath condensate are reduced after 3 wk of treatment with a higher (400 μg daily) but not a lower dose (100 μg daily) of inhaled budesonide (120). In contrast, there is a rapid and dose-dependent reduction in nitrite/nitrate in exhaled breath condensate in the same patients with mild asthma, suggesting that nitrite/nitrate are more sensitive to anti-inflammatory treatment.

COPD. Habitual smokers have unusually high antioxidant concentrations in the epithelial lining fluid and higher resistance to oxidative pulmonary damage. NO can be trapped in the epithelial lining fluid of the respiratory tract in the form of *S*-nitrosothiols or peroxynitrite and released thereafter, leading to transient elevation of exhaled NO after smoking of a cigarette (420). Chronic oxidative stress presented to the lung by cigarette smoke may decrease the availability of thiol compounds and may increase decomposition of nitrosothiols, explaining elevated levels of *S*-nitrosothiols in exhaled condensate in healthy smokers, which are related to smoking history (421). Levels of exhaled nitrite/nitrate are increased in COPD (unpublished observation). A significant negative correlation between FEV_1 and the amount of nitrotyrosine formation has been demonstrated in patients with COPD, but not in those with asthma and normal subjects (422), suggesting that NO produced in the airways is consumed by its reaction with superoxide anion and/or peroxidase-dependent mechanisms, and reactive nitrogen species play an important role in the pathobiology of the airway inflammatory and obstructive process in COPD.

Cystic fibrosis. Elevated levels of nitrite and nitrate (352, 423) and nitrotyrosine (424) have been found in exhaled condensate and sputum (425) of patients with CF during both the stable period and exacerbations. In children with CF and normal lung function, however, the nitrite/nitrate concentrations in BAL are normal and concentrations of *S*-nitrosothiols are reduced (426). In contrast, elevated levels of nitrite and *S*-nitrosothiols are found in exhaled breath condensate of adult patients with more severe CF (427).

Myeloperoxidase, a heme enzyme of neutrophils that uses H_2O_2 to oxidize chloride to hypochlorous acid, is capable of catalyzing nitration of tyrosine, providing an alternative to peroxynitrite in the formation of 3-nitrotyrosine (428). At sites of neutrophilic inflammation myeloperoxidase will nitrate proteins because the cosubstrate tyrosine will be available to facilitate the reaction (428). Patients with stable CF have significantly higher levels of nitrotyrosine in exhaled breath condensate than do normal subjects (424). This suggests that nitration of proteins by myeloperoxidase may be an additional source of nitrotyrosine in patients with CF who have a very low NO production. In fact, myeloperoxidase is elevated in CF sputum and correlates with nitrotyrosine concentrations (425), implying that an absence of an increase in exhaled NO does not exclude the possibility of NO participating in airway inflammation, including CF.

Other lung diseases. Nitrite and nitrate concentrations are increased in exhaled breath condensate of patients with active pulmonary sarcoidosis (214).

Ammonia

Ammonia (NH_3), a product of urease hydrolysis of urea to ammonia and carbamate, is one of the key steps in the nitrogen cycle. Ammonia in the respiratory tract may be able to

neutralize inhaled acid vapors and aerosols, mitigating the pulmonary effects of pollution (429) and has the potential to regulate NOS activity. Thus, plasma of patients with uremia has an inhibitory effect on NOS3 in a human endothelial cell line and NOS2 in murine macrophages (430).

The urea breath test has been in clinical practice for a considerable period of time as one of the most important noninvasive methods for detecting *Helicobacter pylori* infection (76). The test exploits the hydrolysis of orally administered urea by the enzyme urease, which *H. pylori* produces in large quantities. Urea is hydrolyzed to ammonia and carbon dioxide, which diffuses into the blood and is excreted by the lungs. The first measurements of exhaled NH_3 were used to assess different food supplements given during the space flights in the 1970s (431). Recently, using selected ion flow tube mass spectrometric technique the levels of alveolar exhaled ammonia (in the range of 200 to 1,750 ppb) have been detected from single exhalations in healthy volunteers who have ingested a liquid protein meal (432).

Exhaled breath ammonia may be an important counteracting agent in a variety of respiratory conditions, as a low pH in exhaled breath condensate has recently been reported in asthma (32). Exposure to ammonia gas in the workplace is significantly associated with increase in respiratory symptoms and asthma (433). It has been shown that elevated levels of urea can be used to predict oxidative stress, as the levels of urea in saliva are significantly increased after chronic hyperbaric oxygen exposure (434). The fact that acidic rinsing results in a considerable (90%), fast and lasting for 1 h reduction in exhaled ammonia in normal subjects (429) should be considered when ammonia is measured in exhaled condensate.

Ammonia is an important pathogenic factor for certain bacteria, for example *Cryptococcus neoformans*, which is a significant human pathogenic fungus that produces large amounts of urease (435). Exhaled ammonia levels measured by chemiluminescence are not different between normal subjects and patients with stable CF, but are significantly higher in asthma and in normal subjects with upper respiratory tract infections (436). It is possible that measurements of exhaled ammonia might differentiate viral and bacterial infections in a variety of lung diseases.

Electrolytes

Increased airway fluid osmolality in the lower airways as a result of exercise, may activate mast cells and cause subsequent bronchoconstriction in a subset of asthmatics. A deficiency in magnesium and an elevation in calcium concentrations in exhaled breath condensate have been reported in atopic asthma (437), although a histamine-induced decrease in plasma magnesium levels occurs regardless of the diagnosis of asthma (438). We have recently demonstrated that exhaled Na^+ and Cl^- are elevated in exhaled condensates of patients with CF and correlate with the sweat test and the disease severity (Balint *et al.*, unpublished observation). Recently, a strong negative correlation between sputum Cl^- concentrations and exhaled NO has been demonstrated in patients with PCD (191), suggesting that airway mucociliary clearance impairment might be monitored by exhaled/nasal NO and exhaled Cl^- levels.

Hydrogen Ions

An acidic microenvironment up-regulates NOS2 in macrophages through the activation of NF-κB (439), making NO release moderately pH-dependent (30). Elevated levels of lactic acid have been found in exhaled condensate in patients with acute bronchitis (408), and a low pH of exhaled condensate is reported in patients with acute asthma (32). Exhaled pH is free of salivary, nasal, and gastric contamination and is not influenced by either airflow obstruction or inhaled albuterol, but it is increased by corticosteroid therapy.

Proteins and Cytokines

Measurement and identification of proteins in exhaled condensate is controversial. It has been reported that the amount of protein in the breath condensate of eight healthy subjects was from 4 to 1.4 mg, originating from the nasopharynx, oropharynx, and lower airways (347). The same group has also reported the presence of IL-1β, soluble IL-2 receptor protein, IL-6, and TNF-α in exhaled breath condensate of patients with a variety of respiratory conditions (347). Recently, higher concentrations of total protein in exhaled condensate have been found in young smokers when compared with nonsmokers, whereas the levels of IL-1β and TNF-α were not different (440). We have found that IL-8 levels in exhaled condensate are mildly elevated in stable CF but are more than doubled in patients with unstable CF compared with normal subjects (Balint B. *et al.*, unpublished observations).

OTHER METHODS

Exhaled Temperature

Airway cooling provokes an increase in bronchial blood flow and is manifested as a rapid resupply of heat in asthma (441). NO modulates temperature by regulating vascular tone and blood flow (71). Measurements of exhaled temperature and humidity have been used to assess the conditioning function of the respiratory apparatus in asthma, COPD, pneumonia, and pneumoconiosis (442, 443).

Asthma is characterized by inflammation-related vascular hyperperfusion (444), so airway mucosal blood flow and exhaled temperature may be an index of airway inflammation. Indeed, exhaled temperature measured under controlled conditions (standardized expiratory flow and pressure) (56), as breathing pattern may affect airway wall temperature (445), is low in CF and COPD (446), but elevated in asthma when compared with normal subjects (447, 448). Exhaled breath temperature may serve as a nonspecific, simple, and inexpensive method for home monitoring of several upper and lower respiratory conditions such as asthma, COPD, CF, and rhinitis and for assessing the effects of anti-inflammatory treatments.

Combined Gas Chromatography/Spectroscopy

A new analytical method of gas chromatography combined with UV spectroscopy has been used to measure isoprene and acetone in expired air in healthy newborns, preschool children, healthy and diabetic school children (449), or isoprene in healthy adult subjects (450). A new method for analysis of ethanol and acetone in exhaled air using a portable gas chromatograph with a photoionization detector has been developed and has demonstrated that ethanol levels are more than tenfold higher in patients with cardiorespiratory disorders than in normal subjects (451). Exhaled formaldehyde from women with breast cancer and in the tumor-bearing mice is significantly higher than in healthy subjects, suggesting that these carbonyl compounds may be used as a biomarker (452). Laser magnetic resonance spectroscopy (LMRS) is a sensitive and isotope-selective technique for determining low concentrations of gaseous free radicals with high time resolution, which has been successfully used to measure exhaled and nasal NO at the end of exhalation in normal subjects (453), or it can be a simple alternative to mass spectrometry in detection of exhaled ^{14}C-urea in patients with *H. pylori* infection (454, 455).

TABLE 3. CHANGES IN EXHALED GASES IN LUNG DISEASE

	Asthma		COPD		CF				
	Stable	Unstable	Stable	Unstable	Stable	Unstable	Bronch	ILD	PCD
Nitric oxide	↑↑↑	↑↑↑↑	↔	↑	↓	↓	↑	↑	↓↓
Carbon monoxide	↑	↑↑	↑	↑↑	↑↑	↑↑↑	↑↑	?	↑
Ethane	↑↑	?	↑↑	?	↑↑	↑↑↑	?	?	?

Definition of abbreviations: Bronch = bronchiectasis; CF = cystic fibrosis; ILD = interstitial lung disease; PCD = primary ciliary dyskinesia; ↑ = increase; ↓ = decrease; ↔ = no change; ? = not yet known.

The Selected Ion Flow Tube (SIFT) Technique

The selected ion flow tube (SIFT) technique for trace gas analysis of air and breath is based on soft chemical ionization exploiting the ion-molecule reactions that occur between the trace gases and the preselected precursor ions (H_3O^+, NO^+, and O_2^+) (456). This method is sensitive (detection limit is down to about 10 ppb) and fast (response time ~ 20 ms) and can be used during a normal breathing cycle.

Polymer-coated Surface-acoustic-wave Resonators

Portable instruments based on microsensor arrays of polymer-coated surface-acoustic-wave resonators have been introduced and are capable of the analysis of organic vapors (457). Detection of the bound immunocomplex has been made possible via the silicon chip-based light-addressable potentiometric sensor. For example, in the presence of the urea, urease converts the substrate to ammonia and CO_2 and this leads to a pH change at the silicon surface. The resultant pH change can be monitored with time and the signal output can be reported in real time.

FUTURE DIRECTIONS

Exhaled breath analysis has enormous potential as a noninvasive means of monitoring airway and inflammation, oxidative stress, and other conditions (for example, metabolic disorders, bacterial and viral infections). The technique is simple for patients to perform and may be applied in neonates and patients with severe disease. Because the techniques are noninvasive, it is possible to make repeated measurements without disturbing the system, in contrast to the invasive procedures currently used.

Standardization of Measurements

Precautions need to be taken to ensure uniformity of measurement between different centers, and physiologic and measurement factors are likely to differ between markers. This has been most carefully worked out for exhaled NO, and two International Taskforce meetings have defined standards and procedures for measurement of exhaled NO in adults and children (53, 61). Similar standardization methods are now needed for the other exhaled markers currently under investigation.

Clinical Application

There is a pressing need for the evaluation of these techniques in long-term clinical studies (3). Whether repeated measurements of exhaled markers will help in the clinical management of lung diseases needs to be determined by longitudinal studies relating exhaled markers to other measurements of asthma control. This is most advanced with measurement of exhaled

TABLE 4. CHANGES IN EXHALED CONDENSATE IN LUNG DISEASE

	Asthma		COPD		CF				
	Stable	Unstable	Stable	Unstable	Stable	Unstable	Bronch	ILD	PCD
Eicosanoids							?		?
8-Isoprostanes	↑	↑↑↑	↑	?	↑↑↑	?		↑↑	
LTE_4, C_4, D_4	↑	↑↑	↑	?	?	?		?	
LTB_4	↑	↑↑	↑↑↑	?	?	?		?	
PG	?	?	?	?	?	?		?	
Tx	?	?	?	?	?	?		?	
NO-related products							?	?	?
Nitrotyrosine	↑	?	?	?	↑	?			
NO_2^-/NO_3^-	↑	↑↑	↑	?	↑	↑↑			
SNO	↑	↓	?	?	↑	↑↑			
H_2O_2	↑	↑↑	↑	↑↑	?	?	↑	?	?
Lipid peroxidation product	↑	?	↑↑	?	?	?	?	?	?
Vasoactive amines	↑	?	?	?	?	?	?	?	?
Ammonia	↑	?	?	?	?	?	?	?	?
Hydrogen ions (pH)	↔	↑↑	?	?	?	?	?	?	?
Cytokines		?	?	?		?	?	?	?
IL-1_β, IL-2, IL-6,	↑								
TNF-α					↑				
IL-8									
Electrolytes		?	?	?		?	?	?	?
Na, Cl	?				↑↑				
Mg	↓								
Ca	↓								

Definition of abbreviations: H_2O_2 = hydrogen peroxide; IL-1_β, −2, −6 = interleukin 1_β, −2, −6; IL8 = interleukin-8; LT = leukotriene (E_4, C_4, D_4, B_4); NO_2^- = nitrite; NO_3^- = nitrate; SNO = S-nitrosothiols; TNF-α = tumor necrosis factor α. For other definitions, *see* Table 3.

NO (3), but it is still uncertain whether routine measurement of exhaled NO will improve the clinical control of asthma in a cost-effective way.

None of the exhaled markers are diagnostic for a particular lung disease, apart from the very low nasal and exhaled NO in primary ciliary dyskinesia. Nevertheless, measurement of these markers may aid differential diagnosis of lung diseases. For example, a normal level of exhaled NO in a patient with chronic cough makes the diagnosis of asthma very unlikely. A high level of exhaled NO in an asthmatic patient receiving inhaled corticosteroids most likely indicates poor compliance with therapy.

Exhaled markers may also be used to assess the response to therapies such as inhaled corticosteroids and novel anti-inflammatory treatments now in development. Some markers may even be used to predict responses to specific treatments. For example, high levels of LTE_4 in exhaled breath condensates may predict a better clinical response to antileukotrienes, and a high level of markers of oxidative stress may indicate patients who might respond to antioxidant therapy.

Profiles of Mediators

We have reviewed a large body of data on exhaled volatile gases and exhaled breath condensate that demonstrate different patterns of change in different pulmonary diseases (summarized in Tables 3 and Table 4). At the moment single exhaled markers are usually evaluated in isolation, but, as indicated above, markers are affected differently in different diseases, and different markers vary in their sensitivity to certain maneuvers such as the effect of therapy. For example, asthma is characterized by a large increase in exhaled NO, a modest increase in CO, and a moderate increase in exhaled 8-isoprostane, whereas COPD is characterized by little or no increase in exhaled NO, and by larger increases in exhaled CO and 8-isoprostane. By contrast, patients with CF typically have low exhaled NO concentrations and high levels of exhaled CO and 8-isoprostane. Exhaled NO appears to be sensitive to inhibition by low doses of inhaled corticosteroids in asthma, whereas exhaled CO and 8-isoprostane are much less sensitive to inhibition by corticosteroids. These differences may be exploited in the future as more markers are characterized, so that each disease may have a characteristic profile or fingerprint of different markers that may be diagnostic. Treatments too may impose a characteristic effect on these markers, and this may improve the specificity of treatment in the future, particularly as more potent and specific treatments become available.

Measuring Devices

The value of particular markers will depend on the availability of reliable, fast, and inexpensive detector systems. NO chemiluminescence analyzers are currently relatively expensive and are mainly available in academic research laboratories. However, advances in technology have now resulted in smaller devices that are cheaper and easier to use. This will increase the availability of the measurement, which will further reduce the price as exhaled NO analyzers become routine lung function measurements. Eventually it may be possible to introduce such analyzers in family practice and even into patients' homes, so that patients themselves will be able to monitor their own markers and adjust their treatment accordingly.

Measurement of some of the other exhaled markers such as hydrocarbons is much more difficult using present technology, but it may also be possible to develop much smaller and cheaper detectors that would make this measurement more readily available. Although exhaled breath condensates is an attractive approach that could easily be adapted to home measurements, its value is limited by the fact that complex assays, including ELISAs, fluorimetric assays, and HPLC are needed to measure the individual chemical markers. In the future these assays may be simplified by the use of strip reagents that give rapid color changes, so that these measurements may be available for clinicians and for patients to use at home.

New Markers

It is likely that the possibilities for measurement of markers in exhaled breath are far greater than currently realized. It is clear that exhaled breath condensates contain many different molecules, including proteins. In fact, application of proteomics, with high resolution two-dimensional gel electrophoresis and microanalysis of protein spots may allow the recognition of particular protein patterns in different diseases and may result in the recognition of new diagnostic proteins or therapeutic targets. New and more sensitive assays may also allow the detection of many other markers of inflammation and even specific fingerprints of activation of particular cell types within the respiratory tract such as eosinophils, neutrophils, epithelial cells, and macrophages. This could have far-reaching potential for the diagnosis and treatment of many airway diseases.

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PM3006723972